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What should NASA do now?

The fallout from President Ronald Reagan's budget-cutting exercise has been spread more or less uniformly across the Administration on the general principle that all discretionary expenditure — that not decreed under legislation — should be cut by 12 per cent. The National Aeronautics and Space Administration, most of whose expenditure is discretionary, has been by comparison dealt with leniently — its budget is to be cut by only 6 per cent. Yet the exercise on which the space administration is now engaged — egged on by Mr David Stockman's henchmen — is bound to be more painful than in many other agencies. For much of the agency's spending, although technically discretionary, is inescapable. Other agencies, the Department of Defense in particular, will fiercely complain if the much-delayed shuttle programme is further held up. As elsewhere, the space agency also has longstanding commitments to its establishments and (less permanent) to the people who work in them. So when the cuts have been made — a decision is promised for November — the chief casualties are likely to be precisely the parts of the space agency's programme that have been affected by previous attempts to keep the federal budget in check. Space science is the most vulnerable.

The defence of space science is not, of course, a popular cause at this stage in the financial year. Congressmen are likely to be more acutely conscious of the complaints of their constituents than of the long-term value of the planetary exploration on which the space agency has been engaged in the past few years. The abolition of subsidies for school meals will certainly have a wider impact than the threat to the space agency's plans for the remainder of this decade. Yet the issue is not as simple as it seems — esoteric exploration contrasted with the claims of the needy. For there is now a danger that the reductions of the space agency's budget are cutting near the bone. Already the agency has been compelled to abandon its promised part in the venture with the European Space Agency to send a pair of satellites in a polar orbit about the Sun. Now there is talk — mercifully only talk — of cancelling the remainder of the Voyager missions, which would entail that no notice should be taken of whatever they record near Uranus and Neptune. And since (again mercifully) it seems to be agreed within the agency that the Large Space Telescope must be launched as soon as possible, the future for the Galileo mission (to put a complicated satellite carrying a range of planetary probes into an orbit about Jupiter) must now be in serious trouble.

But why should that matter? That is what the people from the White House will be saying. The date fixed for launching Galileo (1985 or 1986) is plainly a movable feast. And will not Jupiter be there for many decades to come? The snag is that the effects of the decision are by no means as simple. For one thing, at least one substantial institution — the Jet Propulsion Laboratory at Pasadena in California — must already be wondering how it will keep itself busy in the thin years ahead. (The Large Space Telescope is to have its own terrestrial laboratory in Maryland.) The obvious danger is that if the laboratory is too far truncated in the months ahead, the capacity of the United States to return to planetary exploration later in the decade will be impaired. Reducing the federal budget deficit is a proper national priority, but should that be allowed to jeopardize other objectives that the United States has enthusiastically embraced in the recent past?

How, in these circumstances, should the agency go about the painful job ahead? The outstanding irony is that the sum of money it has to save — \$357 million out of this year's budget — is a small part of the cost of ploughing on with the development of

the shuttle. The first successful flight of Columbia notwithstanding, and although there can be no denying the interest of other agencies in a successful outcome of the project, few would now insist that this intended workhorse of the United States space programme will be a fully fledged concern in the near future. So the most obvious course to follow is the course that should have been followed more than a year ago — there should be a serious examination of the outstanding problems of the shuttle and, if need be, a serious reconsideration of how best to accomplish the laudable goals that the agency has set for itself. That way, the agency could save the Administration money without harming the rest of its programme. It might even save its own bacon.

When the slump is over

The longer the recession drags on, the more natural it is that people should ask whether it will ever end. But the question is unproductive — logically, it is like asking when the world will adjust to the increased price of oil and then guessing whether, when the adjustment has been made, the price of oil will increase again. Better instead to ask what the industrialized world may be like when the indices of economic growth become positive again. To the extent that many of the governments dedicated to tight monetary policies seriously intend that the consequences should include new patterns of industry, the question is moreover not idle. Most of these governments hope that newer industries, loosely called electronic, will be more prominent in the eventual mix. So will the clouds lift to reveal everybody working either with electronic equipment or on its manufacture (software included)? The short answer is that the world will not be like that. Some of the reasons have been well put in, or can be read between the lines of, the sixth of a series of reports on the information industry published by the Organization for Economic Cooperation and Development (OECD)*.

The truth is that people cannot eat or drink electronic equipment, shelter beneath it or even (except in the most conceptual sense) use it for travelling about. Even in the past decade, when the variety and quality of electronic equipment on the markets of the West has turned most shopping streets into Aladdin's cave, purchases of these wonders by consumers have not substantially increased as a proportion of all personal expenditure. Comparisons between countries are complicated by the difficulties of definition, and most of the figures are in any case out of date. But between 1950 and 1978, in West Germany, the proportion of consumer expenditure on electronic equipment actually declined, to between 6 and 7 per cent of all consumer expenditure. In the United States (using a broader definition of "information" and related activities), the proportion of consumer expenditure on such activities is stagnant at about 17 per cent. In Japan, the equivalent proportions are larger and still growing. But everywhere, consumers' priorities are more or less unaffected by the coming of the electronic revolution. Housing and household goods come first, food and drink second and travel third. It will be a long time before the information industries catch up.

Inevitably, then, the OECD report concludes that there is as yet

*Information Computer Communications Policy 6, *Information Activities, Electronics and Telecommunications Technologies* Vol. 1 (OECD, Paris, 1981).



no evidence that the electronic revolution has had an important influence on the volume of consumer demand. For the time being, ordinary people are more concerned to buy themselves what they regard as the necessities of life than the new-fangled equipment they see in the shops. The implications are straightforward. At least while prosperity is constrained by the recession, the products of the information age will have to make their way in the market by displacing other goods from consumers' budgets. And even when prosperity returns, people will go on wanting houses to live in and food to eat. People with ambitions to sell a domestic computer to every household will have to make their sales at the expense of other commodities — and probably at cut-throat prices. Only over much longer periods of time, perhaps as people's habits change under the influence of travel costs, are these priorities likely to change. Inevitably, electronic manufacturers will find that demand for their products increases rapidly as the recession draws to an end, but even then they will not be able to capture more than a comparatively small share of what consumers spend.

Why then are manufacturers of electronic equipment irrepressibly optimistic? The simple explanation is that there is every reason, even now, why business and manufacturing industry should use electronic and information-processing equipment to simplify and cheapen their operations. Especially because restrictive monetary policies have often had the effect of increasing the cost of employment, the substitution of people by electronic machines is likely, in the months ahead, to gather pace. Already there is telling evidence of the magnitude of the savings that can be made. In the Japanese colour television industry, for example, output increased by a quarter between 1972 and 1976, while the numbers of people employed on making them decreased by 40 per cent. For the past several years, there has been a running argument, in which trade unions have played an important part, about whether new developments in electronics will lead to permanent and irremediable unemployment. It seems now to be well-established that this need not be the outcome. To the extent that the information industry cheapens industrial costs and ultimately the costs of goods and services to consumers, demand for novel products should increase and opportunities should be created for more employment. The trouble is that re-employment is likely to lag behind unemployment, people's capacity to find new jobs is compromised by their lack of training and, in any case, there is little prospect that new jobs will materialize if new industrial investment is at a low ebb. Quite properly, OECD shades the conventional wisdom that increased automation is an opportunity and not a threat with the sombre reflection that this may not be the case in the years immediately ahead. Equally properly, it argues that further restrictions of the length of the working week will hinder, not help, if the result is to make one country less able to stand up to external competition.

Nobody can tell, as things are, how much of unemployment in Western Europe and North America can be blamed on the doings of the electronics industries in the past few years. The OECD guess is that the proportions are still only small. Monetary policies, whose effects are broadly calculable, are sufficient to account for what has been happening. But this may change when the dole queues lengthen. And since consumers will continue to spend a substantial part of their incomes on their traditional priorities, there will not in the end be a lot of slack to be taken up by entirely novel industries. So what will be the consequence? Most obviously, not all the governments now seeking to foster domestic electronics and information industries can hope they will succeed. For better or worse, international trade ensures that, especially in these new fields, competition is effective. Government delegations to Tokyo can hope to blunt the edge of competition temporarily, but such advantages cannot persist. It is a better strategy to plan for a reasonable chance of success — the provision of more and better training for example (which the British government, for one, is shockingly neglecting). Communities that lose in this competition will find it necessary to produce, instead of hardware and software for the new industries, traditional goods and services that other people want to buy.

Paradoxically, for some the most noticeable effect of the changes in the pattern of industry will be the need to grow or to process more food.

Cheers for neurobiology

Did somebody use his influence with the Nobel Committee in Stockholm to ensure that the announcement of this year's prizes in physiology and medicine should appear on the eve of next week's jamboree of the American Society of Neuroscience in Los Angeles? Certainly it should further serve to enliven what is usually an ebullient meeting that the prize should have gone to three distinguished neurobiologists — Dr David Hubel and Dr Torsten Wiesel for their mapping of the structures of the visual cortex and to Dr Roger Sperry for his several distinguished contributions to the understanding of the brain. The Nobel citation of his work with animals in which the two hemispheres of the brain have been separated, which first helped to make possible an understanding of hemispheric specialization, and which has also forged links between nervous anatomy on the one hand and psychological and clinical studies on the other, is only one of those that might have been chosen. And nobody, of course, disputes the importance of what Hubel and Wiesel have jointly and separately accomplished; their work is both monumental and, more generally, most probably a guide to other architectural principles in the brains of vertebrates. Everybody will be glad that so much has at long last been recognized.

As in previous Nobel seasons, however, there are also regrets. With the best will in the world, the Nobel committees cannot hope to practise absolute fairness. In the old days, indeed, it seems that the search for judicial impartiality was tinged with more than an element of partiality — R.M. Friedman's account of the troubles over Einstein's prize in 1921 (see *Nature* 292, 793; 1981) is a compelling account of how the old committees saw the world their own way. Nowadays, given the temper of the scientific community, the rapid spread of the news of discovery and the recognition of the importance of the prizes as such, those involved with the selection process most probably do do their utmost to be fair. Yet the decisions are still puzzling. If Hubel and Wiesel deserve a prize in 1981, for example, why could this not have been recognized several years ago, when the importance of what they had accomplished was already plain — and when the freshness of their work, if quickly recognized, might have been even more stimulating for others? And how, in any case, did the Nobel committees distinguish between this work on the visual cortex and that of V.B. Mountcastle, who was the first to demonstrate columnar structures in the somato-sensory cortex?

Fine distinctions such as these are never easy. They inevitably contain an element of rough justice. And there is every reason to expect that recipients of the prizes will go out of their way, whenever that is appropriate, to acknowledge what others have independently accomplished in similar fields. The problem is one for the Nobel Foundation (and for those who advise it); certainly it should not be a difficulty for the recipients. Its essence is that some way should be found of tuning the impact of the Nobel prizes more accurately to the present climate in the scientific community without putting at risk the value of the prizes as stimuli to chosen fields of investigation and as marks of honourable achievement. Several ways in which the foundation might change its ways are obvious, of which the most urgent is that the exclusive terms of Alfred Nobel's will should not be taken too literally (see *Nature* 287; 668; 1980). Fortunately there are rumours that some reform along those lines is in the wind. Awarding prizes in part at least to institutions would also help, but perhaps the most glaring anomaly to have emerged since the beginning of the century is that the numbers of prizes awarded have not kept pace with the growth of the profession, even with the now almost invariable practice of awarding prizes to a group of people, not a single individual. The solution may be to go even further along this road, for there is a long way to travel before the currency could be debased by being too plentiful.

UN environment programme threatened

Officials urge withdrawal of US support

Washington

The future of the United Nations Environment Programme (UNEP) has been placed in jeopardy by the decision of top officials in the US State Department to recommend withdrawal of all US funds for the programme from the beginning of October. Environmentalist groups and others are lobbying hard to prevent the State Department from officially endorsing this position, since the \$10 million which the United States contributes annually to UNEP is about a third of its total budget.

The budget cuts are a direct result of President Reagan's decision three weeks ago to demand an immediate across-the-board cut of 12 per cent in so-called discretionary spending for all federal agencies.

A resolution passed by both the House of Representatives and the Senate at the end of October — the stop-gap measure needed to ensure that government departments continue operating even though their budgets for the fiscal year 1982, which starts on 1 October 1981, have not yet been agreed to — contained a 12 per cent cut in the \$215 million allocated for voluntary contributions to international funds by the State Department. Documents apparently drafted by officials of the Office of International Organizations recommend that, rather than distribute these cuts across all agencies funded out of this budget category, the reductions should be made by completely eliminating contributions to particular funds.

Among those recommended for elimination is the \$7.5 million which was to have contributed to the Interim Fund for Science and Technology, set up following the UN Conference on Science and Technology for Development in Vienna in 1979. There seems little likelihood that this contribution will be rescued, partly because there is no significant constituency in Washington lobbying for the Interim Fund. The outlook is equally bleak for two other contributions, one for a Trust for South Africa, the other to support UN activities in Namibia.

The proposal to cut UNEP funding could run into stiffer opposition. Representative Don Bonker, chairman of the human rights and international organizations subcommittee of the House of Representatives Foreign Affairs Committee, has already written a letter of protest to Mr Elliot Abrams, Assistant Secretary of State

for International Organizations, and is to meet Mr Abrams this week. One of the points which Mr Bonker is expected to make is that a move which could eventually lead to the dismemberment of UNEP as an independent agency could have repercussions for the United States not only

in its relations with other industrialized nations, such as Sweden, which support the programme, but also with developing nations. UNEP, based in Nairobi, Kenya, is the only UN agency to have its headquarters in a Third World country.

David Dickson

Patents to mean slow publication?

Washington

The fear that merely submitting a manuscript to a scientific journal for possible publication could jeopardize the chance of obtaining subsequent patent protection in some European countries has prompted four US federal agencies to require notification of any potentially patentable research results at least three months before they are submitted for publication.

This rule is the result of a circular issued by the Office of Management and Budget (OMB) in July suggesting how it might apply the terms of a new patent law, covering federally sponsored research carried out in universities and small businesses, passed by Congress at the end of last year. The rule has already been adopted by the Department of Energy, the Department of Defense, the National Aeronautics and Space Administration and the Environmental Protection Agency.

The proposed rule has already created a storm of protest from the US research community, which claims that, by threatening to deny a scientist patent rights to a discovery if the procedure is not followed, it could seriously impede scientific communication.

In its final form, to be issued by OMB before the end of the year, the bill is likely to be at best a compromise between scientists who feel that they should be free to try to get a scientific discovery into print as soon as possible, and federal administrators who fear that excessive zeal to publish could jeopardize foreign patent rights.

The July circular from OMB says that all federal agencies will have the option of requiring those scientists they support to notify the agency of patentable research three months before a manuscript is submitted for publication in a professional journal. According to OMB's Office of Federal Procurement Policy, agencies favouring this requirement feel that it is necessary to assure protection of foreign patent rights, particularly when national security interests are involved. But the National Science Foundation (NSF) and the National Institutes of Health (NIH) have already indicated that they have no intention of exercising this option, leaving scientists to inform their funding agencies of results at the same time as they submit them for publication.

Dr Howard W. Bremer, patent counsel

for the Wisconsin Alumni Research Foundation — one of the most successful university-based licensing groups in the United States — claimed that the OMB statement that merely submitting research findings to a scientific journal represented a form of disclosure that could act as a statutory bar in some European countries to later patenting was "wholly without foundation".

Mr James Denny, assistant counsel for patents at the Department of Energy and chairman of the interagency group responsible for drawing up the proposed regulations, disagrees. The problem would not apply in the United States, where a patent can be applied for up to one year after publication of research results, but under the new patent rules introduced by the European Economic Community, scientists might be unwittingly forfeiting their chances of a European patent.

At NIH, plans are already being drawn up to implement the government's new patent policy, which is essentially an extension to all universities and small businesses of the Institutional Patent Agreements which individual agencies such as NIH and NSF had previously negotiated with a limited number of research universities.

There are other worries resulting from the new patent laws. Several members of a subcommittee of the Advisory Committee to the Director of NIH, looking at co-operative research relationships with industry, have expressed concern about the condition proposed by OMB that for research jointly funded by the federal government and a private sponsor, the patent should remain the property of the government, however small its contribution to the research.

If rigidly interpreted this might scare off potential corporate supporters, but OMB's associate administrator Mr Fred Dietrich says that the provision would be invoked only where there had been a clear transfer of potentially patentable ideas, for example from a scientist working on the federally sponsored part of a project to one working on the corporate-sponsored part. All of which suggests that researchers will certainly have to keep meticulously detailed records of who paid for the various aspects of their work.

As a result of the new patent law, NIH will in future require anyone receiving a grant to sign an agreement stating that one

of the conditions of the award is that the agency be informed promptly if any potentially patentable results emerge from the research.

David Dickson

EEC science policy

Grand Plan to flop?

Brussels

The European Commission is preparing a grand strategy on the European Community's research policy for the 1980s which is to be the basis of a Council of Science Ministers on 9 November.

The policy paper, which is still undergoing revisions, has been put together under the direction of Viscount Etienne Davignon, the European Commissioner who has the main responsibility for scientific affairs in Gaston Thorn's Commission. Davignon has chosen to continue with the basic priorities that have been the staple diet of past community policies: energy research, particularly fusion and nuclear safety, environmental protection, raw materials and the coordination of indirect action programmes.

There are some changes though. Davignon wants to bring together under his direction all of the Community's research and development programmes, including agricultural research, research and development for the use of developing countries and data processing. Some see these proposals as examples of Davignon's ambition, but he seems more concerned with creating more efficient administration.

Davignon intends to do away with many of the advisory and expert committees which clutter up the decision-making

process. But to achieve greater flexibility in decision-making, Davignon suggests that the Council of Ministers should set up a standing committee.

The council in November will consider fixing the Community's research activities up to 1986. To this end, Davignon suggests a budget which would progressively increase by 2 per cent a year. This is well below the inflation rate in any of the ten member countries and thus represents a decrease in expenditure; however, it bears comparison with expected increases in national research budgets.

Davignon will be pushing for increased expenditure in a few fields. He would like more spent on nuclear fission research and industrial innovation. In this he has the support of the two other commissioners involved, the German Karl-Heinz Narjes with responsibilities for industrial innovation and nuclear safety, and the Englishman Ivor Richard who has responsibilities for education and social affairs. Davignon is especially keen for the Commission to take a lead in promoting nuclear safety research in areas not covered by national programmes. The aim would also be to lessen public mistrust of nuclear power.

The French, having taken a decision to go ahead with the old regime's nuclear power programme and increase the entire national research budget, will probably support the new plans. The rest of the Community is doing its utmost to reduce public expenditure in all fields. There are strong signs that some governments regard community research as a luxury — for example, the Dutch want to reduce their direct contribution to the EEC research centre at Petten.

Jasper Becker

Energy in France

Small change

President Mitterrand's honeymoon is over. His government won the vote in the National Assembly last week for a large nuclear power programme (six new reactors to be begun in the next two years), but only at the cost of opening up divisions in the Socialist Party and calling into question its electoral promise of "a new politics" of energy.

M. Paul Quilès, socialist deputy for Paris and author of the party's electoral energy report, has been trounced; and a member of ex-President Giscard's party described the new energy policy as similar to Giscard's — only with less courage.

On just one point, M. Quilès won a concession: the restarting of construction at the reactor sites where work was "frozen" a few months ago, pending the definition of government policy. Before these sites are reopened, local authorities will be consulted.

However, the affected local authorities — at Cattenom, Golfech, Chooz, Civaux and Le Pellerin — will have to move fast. They have less than a month to say yes. If they say no, the decision will move up to the regional assembly concerned. If, within the same month, this assembly cannot agree or choose a new local site, the decision reverts to parliament and government — which then have to decide if the construction is in the essential national interest. If it is, the reactor goes ahead regardless.

There are other differences between the Giscard and Mitterrand plans. Giscard wanted to start eight reactors at 1,300 MW

UK universities redundancy plans

As the vice-chancellors of British universities struggle to draw up a national redundancy scheme for academics, more universities are estimating the number of posts that will have to go over the next three years. Many hope that economies for the current year can be made by cutting recurrent expenditure, by voluntary redundancy and by early retirement schemes. They plan to stave off compulsory redundancies until 1982–83. Others do not enjoy that luxury.

The universities of Stirling and Aston in Birmingham, for example, both among the most severely penalized, expect to move quickly. Stirling's problems are complicated by the low average age of its academics, which means that there are few candidates for early retirement. Among the less heavily cut, however, there are also some surprises. The University of Bristol, for example, says it must lose 400 posts over three years, 150 of which it have to be met

by compulsory redundancy.

The vice-chancellors' plan to draw up national guidelines for compulsory redundancy is hampered by the large variety of contracts of employment for academics. They hope to have more concrete proposals after their next committee meeting at the end of the month. But any plan will not be welcomed by the Association of University Teachers (AUT), the academic trade union, which does not acknowledge the need for compulsory redundancy. AUT is prepared to fight cases in the courts as soon as they arise, saying that it will contest decisions to sack academics even before individuals have been named. In that case, arguments will rest on independent assessments of a university's finances and the conditions of employment for academics laid down in its charter.

The accompanying table gives the number of full-time equivalent jobs that the universities estimate will have to be lost over the next three years. Some estimates are firmer than others, but few at this stage show how many posts can be lost voluntarily.

Judy Redfearn

Estimates of full-time post losses at selected universities

University	Academic	Non-academic	Total no. of existing posts
Bristol	259	141	3,500
Hull	110	100	2,000
Sussex	78	157	1,272
Aberdeen	300–350*	*	2,696
City	60 (314)	90	552†
Aston	150 (600)	300	2,000
Stirling	60 (260)	120	1,100
Salford	200	300	1,500
Bradford	150–180 (495)	—	—
Exeter	70 (500)	—	1,900

Figures in parentheses give the current number of academics.

*Includes both academic and non-academic posts.

†Academic, clerical and technical posts.

and one at 900 MW in 1982–83. But since then, Electricité de France has revised downwards its electricity demand projections for 1990. The new government's target of six 1,300 MW reactors and one 900 MW reactor is commensurate with the new projections.

More identifiably new are proposals for public and regional consultation over nuclear decisions, and the establishment of several new energy committees. But, in the end, power seems likely to rest firmly at the centre.

There will be an "observatoire de l'énergie" at the Ministry of Industry, to act as an information bank; a "regional energy agency" under each *Assemblée Regionale*; and adversary, representative local information commissions set up on each power station, reprocessing or fuel assembly site.

The regional energy agencies will also be representative — consisting mostly of elected members, together with some union, public and interest group representation — and their first enormous task will be to prepare a full regional energy plan. These councils will consider local economic, demographic and geographical factors and devise a plan of action — including a politics of information, research, development and financial support.

Other plans outlined are for the National Assembly to set up an office of technology assessment and for a permanent delegation for energy at the assembly, to consider matters of safety and security. The expert *Conseil Supérieur de la Sécurité Nucléaire* will be reinforced and its composition "modified".

On the remaining nuclear questions — reprocessing and the fast breeder — the French government is allowing itself only a little more time. Construction to double the capacity of the existing plant at Cap de la Hague is due to begin next March; and in the interim a special scientific commission will investigate all questions concerning reprocessing (including the poor functioning of the present plant), and report to government and parliament. *Superphénix*, the commercial demonstration fast reactor at Isère, will be started up; but decisions on the commercial development of the fast breeder will be deferred to 1984–85.

Meanwhile, Quilès and his many erstwhile followers in the socialist party feel let down. Although none goes quite as far as M. Michel Rocard, leader of a socialist party faction and previously a contender for the presidency. "What's happening to the party is an historical scandal" a Paris paper reported him as saying last week. "Never in the international workers' movement — including Lenin's time — has there been such an attempted internal putsch so cynical and so empty of principles." M. Rocard, it seems, was not referring solely to energy policy.

Robert Walgate

Keyworth takes on health risks

Washington

In a small but potentially significant realignment of Washington responsibilities, the new director of the Office of Science and Technology Policy, Dr George (Jay) Keyworth, has agreed to be the chairman of a group which is being established to coordinate the scientific aspects of federal policy on safety, health and environmental regulation.

Under the Carter Administration, this coordination function was carried out by the Interagency Regulatory Liaison Group (IRLG). Chaired by the then administrator of the Environmental Protection Agency, Dr Doug Costle, IRLG provided a forum in which federal agencies could coordinate their efforts on common or overlapping problems. In 1979, for example, IRLG published a report on the scientific bases for identification of potential carcinogens and estimation of risks prepared by a working group with representatives of four regulatory agencies and headed by Dr Eula Bingham, then head of the Occupational Safety and Health Administration. This group agreed that any substance shown to cause cancer in laboratory animals should be treated as a potential human carcinogen.

The new heads of the regulatory agencies, appointed by Mr Reagan, have adopted a substantially different approach to their mandate, emphasizing ways of cutting back on health and environmental regulation rather than adding to it. They have decided to let IRLG disappear, but they have agreed to support a new group, provisionally entitled the Interagency Science/Health Coordination Group, chaired by the President's science adviser, Dr Keyworth.

Dennis Prager, OSTP's assistant director for life sciences and institutional relations, explained that the working group set up under Vice-President George Bush to look at ways of reducing the burden of regulation on private industry had recognized the need for both a scientific contribution their discussion and for continued interagency coordination, and had felt that "the best way to do that was to upgrade the interagency council and give it more clout by being chaired by Jay Keyworth." Mr Prager said that the group would be trying to strengthen the scientific basis for regulatory decisions and develop methods of risk assessment.

David Dickson

Conflicts of interest

Tighter controls

Washington

Leading research universities across the United States are suddenly having to tighten up the rules which cover potential conflicts of interest between the academic and commercial activities of individual research workers. Their efforts stem from the growing need to order the complex web of relationships caused by the rapidly increasing ties between university and corporate research efforts.

In most cases, the demand for tighter conflict-of-interest rules has come from within the universities. Two weeks ago, for example, the faculty council of Harvard University agreed to recommend to the full faculty a new set of procedures which would establish a Faculty Committee on Conflicts of Interest. If adopted, the procedures would, for the first time, require certain faculty members to disclose details of their outside commitments, as well as formally restricting such commitments to 20 per cent of their time.

There has also been pressure for stricter control from outside. In California a federally-funded public interest group, California Rural Legal Assistance (CRLA), has demanded that research workers at the University of California should no longer be exempt from a law requiring all state officials to disclose their economic interests. Last Monday the

state's Fair Political Practices Commission agreed that CRLA's staff should look into alternative regulations for university employees.

The debate on how a scientist should strike a reasonable balance between his or her teaching and research commitments to the university and those as consultants for outside companies is not new to US academic life. However, it has been given a new twist by three particular elements.

Two spring directly from recent changes in federal policy. The first is a set of legislative measures, such as tax incentives and patent law reform, designed explicitly to tighten the links between the academic and the corporate world. The second is the growing squeeze on federal research funds, inevitably leading university scientists to seek corporate sponsorship with greater zeal.

At Harvard the need for new guidelines was highlighted last year when the university's president, Dr Derek Bok, made a proposal which he subsequently retracted — that the university should have an equity share in Genetics Institute, a company being established on venture capital funding by Dr Mark Ptashne, professor of molecular biology.

Harvard's new rules would require that faculty members consult with a new committee, chaired by the dean of the faculty of arts and sciences, about any activities that "seemed to present an unacceptable conflict of interest or commitment". These include cases where a

faculty member takes on executive responsibility for an outside corporation, makes use of unpublished findings from university research in outside projects, or takes to another location research which would ordinarily be carried out on campus.

University administrators are pains to point out that such guidelines will be interpreted flexibly. Dr Walter Gilbert, for example, has recently been given leave of absence to work as chief executive officer of the Geneva-based Biogen, but will be able to return to his post as professor of molecular biology at a later date.

Stanford University, which has more formal guidelines than most universities because of the involvement of its faculty members in the microelectronics industry, is also tightening them up. The faculty will meet next month to decide whether to adopt a set of proposals that would require any research worker to disclose the names of companies with whom he or she consults and the nature of the consultancy agreement to the appropriate dean or head of department.

The Stanford proposals, made public in the early summer, are also designed to ensure that neither faculty members nor the departments to which they belong should make fortunes from the licensing of patents; academics will usually receive a third of any profits, but departments will have to share with the university if their income exceeds a certain proportion of their budget.

A different, although closely related, set of issues has emerged at the Massachusetts

Institute of Technology (MIT), where Mr Edwin (Jack) Whitehead, founder and former president of Technicon Corporation and now the largest shareholder in Revlon which recently bought Technicon from him, has offered to build and endow an independent institute for research into molecular approaches to developmental biology (*Nature* 1 October, p.329) at a total cost of \$127 million.

Although strongly supported by the MIT administration, the proposal has generated considerable controversy among faculty members over the implications of the possible "dual allegiance" of the 20 new professors who would be appointed to the MIT faculty but have their salaries and research expenses totally paid for by the Whitehead Institute. At a meeting on 1 October during which several criticisms of the proposed arrangements were raised, faculty members called on the MIT administration to take their concerns into account in further negotiations.

Meanwhile conflict-of-interest concerns are even reaching the top ranks of the mass media. Mr Walter Cronkite, until recently anchorman of CBS's nightly evening news, announced on Friday that he was resigning as a director of Pan American Airways since this could conflict with his work on space topics for the new CBS science program *Cosmos*. Earlier this year Mr Cronkite withdrew from taking part in CBS's coverage of the first launch of NASA's space shuttle, a project for which Pan Am has contracts. **David Dickson**

University organization London gives up

The committee set up a year ago to devise a new structure for the University of London's non-medical schools is being scrapped. Last week, Professor Randolph Quirk, the university's new vice-chancellor, explained in a letter to Sir Peter Swinnerton-Dyer, the committee's chairman, that the worsening of the university's finances over the past 18 months made the committee's original brief virtually impossible. Since the committee produced its first discussion document last January, the university's financial prospects have changed from a 15–20 per cent cut in real income by the end of the decade to a 20 per cent cut over the next three years.

The response to the committee's interim recommendations last May was mixed. In particular, suggestions that Chelsea College should close and Bedford and Westfield colleges should merge met with protests. Academics may be relieved at the committee's demise, but their respite will be brief. Professor Quirk hopes that the subject review committees, which he set up last month to advise on resource allocation by January, will establish criteria on which the university can determine its future for itself. The review committees will assess the quality of teaching and research in biological, physical and engineering sciences, languages and social studies in all colleges of the university.

The Swinnerton-Dyer committee, however, has been asked to report by December on the university's central activities, including research and teaching institutes, the central library, unions and sports facilities and central administration. The idea is that the committee's work will form part of the information used to help allocate future resources.

Meanwhile, the university is still struggling with an even older problem — that of rationalizing its medical schools. The recommendation in early 1980 of a working party chaired by Lord Flowers that London's 34 medical institutes be merged into six large schools was protested at more strongly than the Swinnerton-Dyer proposals.

Lord Flowers' grand plan has since been superseded by piecemeal decisions on the fate of individual schools. Hence St George's Hospital Medical School is to increase its intake of preclinical students from 200 to 250 by 1985, the preclinical courses at Westminster and Charing Cross Hospital medical schools are to merge, the Royal Free Hospital Medical School is to remain much as it is and Guy's and St Thomas's Hospital Medical Schools are to merge.

Later this month, university officials will meet to discuss whether to merge the three large medical schools in north central London — St Mary's, the Middlesex and

Solidarity for university freedom

The transformation of the Polish free trade union Solidarity into a multilateral social movement was completed at its first congress in September–October. The congress in Gdansk discussed a wide range of proposals and programmes and in particular called for a new approach to education, involving "social control" over the whole educational process.

The formal resolution derives from the report of a special working group which, among other things, advocated liberalization and self-government in higher education, an end to censorship and free access to the works of Polish scholars living abroad.

It also called for university and research finances to be effectively under the control of "academic and social milieux". Scientific exchanges with foreign scholars should be increased, and more funds made available for the purchase of books and learned journals from abroad. Just how this could be accomplished in Poland's present economic crisis is hard to see — although Solidarity does, of course, have its own proposals for economic recovery.

One interesting suggestion is that a new system of "social funding" of research

and higher education should be developed, in parallel with the government sector. On the Polish Academy of Science which, it is suggested, is too isolated from the rest of the academic community, the group advocated the removal of all "barriers" with the academic community, with the understanding that scholars working for the academy should be able to give lectures and seminars for students.

The group also asks that the status and authority of scholars should be restored by solving the "personnel problem" in various universities, a reference to those academics who owe their positions to patronage rather than academic prowess. Academic degrees should be awarded by competent scholars who enjoy the full trust of their fellows — without, it is implied, the possibility of government or party influence.

The most startling proposal raised by the working group, however, is that Solidarity should found its own private university in Szczecin, taking as a precedent the Catholic University of Lublin. On this point, however, the working group failed to reach a consensus, and the proposal was deferred for further study. **Vera Rich**

University College hospitals and where to accommodate a new preclinical school for St Bartholomew's and the London Hospital.

The ultimate aim is to save £3 million in recurrent grant spent on medical education and to eliminate an excess capacity of pre-clinical places. As yet, the university has no clear estimate of how near it is to achieving that goal.

Judy Redfearn

Water and the Third World

New agency planned

Bangalore

Acute, recurring shortage of potable (drinking) water in India has forced the government to set up a national water development agency charged with the task of surveying and sterilizing the water resources in the country scientifically. The agency, to be called Rashtriya Jal Vikas Abhikaran, will have as its members chief ministers of 21 states in India.

This development comes ironically at the start of "water decade" when the quest for clean potable water has become paramount. Nearly 30 per cent of the diseases and deformities in the Third World can be directly related to polluted water.

India is currently utilizing only 15 per cent of its surface water because of the lack of appropriate and economically efficient measures for harnessing the peninsular rivers and streams. However, efforts are under way to draw-up schemes for modernization of irrigation systems. In India, the level of production from irrigated areas has remained low, at less than half its realizable potential. The Indian irrigation department say that the irrigation potential in the country is already 59 million hectares and it is proposed to create another 54 million hectares by the end of the century.

The water development agency will also consider ways of increasing the efficiency of water use, the maintenance of existing canals, and a flood control programme.

B. Radhakrishna Rao

● In Europe the question of exporting technology and expertise to help the Third World in its quest for clean water is being taken very seriously. Tom King, UK Minister for Local Government and Environmental Services has praised British consulting engineers, contractors and equipment suppliers for their exporting ability — exports of water supply plant and equipment alone totalled £51 million in 1980. Prospects look good with schemes such as the Cairo sewerage network and the Khark project in Iraq in the offing. This British expertise has also been examined by Michel Crepeau the French Minister of the Environment during his recent visit to London and the Thames Water Authority. "The Third World depends on water like we depend on oil", he said, in stressing the French interest in the "water decade".

Sara Nash

US nuclear power

Go for Clinch River

Washington

In a widely-anticipated reversal of the policies of President Jimmy Carter, the Reagan Administration has announced that it is lifting the ban on the commercial reprocessing of commercial nuclear waste, and will allow construction work to begin on the liquid metal fast breeder reactor at Clinch River in Tennessee.

The ban on reprocessing, and the delay in the construction of the fast breeder, had been imposed by President Carter in the hope of steering other industrialized nations away from a "plutonium economy" which would, he claimed, inevitably increase the risk of the proliferation of nuclear weapons.

Neither argument, however, is supported by the Reagan Administration. A policy statement released by the White House last Thursday repeated the Administration's promise to accelerate the licensing of new nuclear power plants, virtually at a standstill since the Three Mile Island accident of 1979, and to push for an early solution to the problems of radioactive nuclear waste disposal.

Dr Nunzio Palladino, the new chairman of the Nuclear Regulatory Commission, said that the Administration was now studying whether or not it would be necessary to introduce new legislation to speed up the licensing process. Even without such legislation, the commission was planning to grant 33 new nuclear plant operating licences by the end of next year. Much depends on building work.

The White House laid the blame for the licensing delays squarely at the feet of excessive federal regulation, claiming that the morass of regulations "is forcing many utilities to rule out nuclear power as a source of new generating capacity".

Several Democrat congressmen have attacked the Administration statement for supporting, in the words of Representative Richard Ottinger of New York, "a Chrysler-type bail-out that will cost the taxpayer billions of dollars". There is some sympathy for this view within the Office of Management and Budget, whose director, Mr David Stockman, has been generally reluctant to exclude the nuclear industry from his general opposition to government subsidies for the private sector.

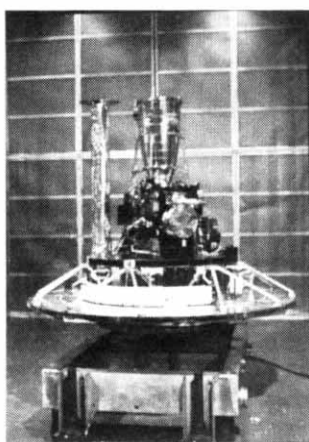
Conflict between conservative budgeteers and the nuclear industry is coming to a head over whether the government should provide such subsidies for the development of commercial reprocessing facilities for spent nuclear fuel or whether this should be left to private enterprise, which seems reluctant to take on this responsibility in the light of current low levels of demand.

It also seems unlikely that the last has been heard about the Clinch River fast breeder. Earlier this year, Congress, which had managed to prevent Mr Carter closing down the whole project, voted money to allow the start of construction, a position now endorsed by the White House.

Last week, however, 25 prominent members of Congress called on Senate leader Howard Baker to withdraw his support for the project and ask for its cancellation on the grounds that it was difficult to defend the \$3,000 million which the Clinch River fast breeder will cost to build when other areas of the federal budget were being squeezed so tightly. Senator Baker represents the state of Tennessee, and his support for the reactor is said to have been the main reason for the Reagan Administration's endorsement.

The decision to lift the ban on reprocessing is being seen as primarily a domestic decision, even though President Carter made it clear that the ban itself was something he hoped other nations would copy.

David Dickson



The Solar Mesosphere Explorer (SME) satellite (left) was successfully launched on 6 October by a NASA Delta rocket (right) together with the "amateur" satellite UOSAT (built by a team from the UK University of Surrey). Both satellites achieved their planned orbits. The SME satellite will investigate processes affecting the distribution of ozone in the Earth's upper atmosphere. (For a full description of the mission, see *Nature* 24 September, p.259.)

CORRESPONDENCE

Animal pain

SIR — Your editorial on "Protection for laboratory animals" (*Nature* 17 September, p.173) is most welcome. It is no longer enough for universities and research workers to maintain an aloof silence or to claim that they are working within the law. Public opinion will not accept such an attitude. I am sure that there are many scientists who regard themselves as animal lovers, or even just humane people, but believe sincerely in the benefits that animal experiments have brought to mankind and to animals themselves. Perhaps we should begin to speak out and not accept false propaganda on the one hand or the sanctity of all knowledge on the other.

A first step would be to show that scientists do care about animals and do think deeply about what they propose to do, the best way of doing it and just why they are doing it. Your proposal to set up committees at many centres is timely. Indeed, committees of this sort have already been formed at several universities and are under discussion elsewhere. If such bodies were made responsible for approving applications for licences and if necessary interviewing applicants, this alone would help to ensure that the work is not unnecessarily repetitious, that an excessive number of animals is not used and that alternatives have been considered. Such advice would be particularly helpful to inexperienced workers who often receive only very limited and specialized training.

Is it not time that all new licence holders were asked to attend a short formal course on animal welfare, breeding, feeding, preoperative and postoperative care, anaesthesia, use of drugs etc.? After all, if public money can be spent on sending general practitioners to courses on intrauterine memory, would the public not agree that funds should be available for training biologists in the basic practical care of those who are used to benefit mankind but are given no choice in the matter?

R. BARER

Department of Human Biology and Anatomy,
University of Sheffield, UK

SIR — Your leading article "Protection for laboratory animals?" (*Nature* 17 September, p.173) mentions "widespread incoherence", and may I respectfully suggest that a number of misleading statements have hardly clarified the situation. For instance you say, "Thus the 1876 Act requires that experiments involving pain must be carried out under anaesthesia (and the Home Secretary has not waived this regulation for the past half-century)". Yet, according to Home Office Statistics of experiments on living animals (Great Britain, 1980), 3,730,588 experiments, "calculated to inflict pain", were carried out without any anaesthetic (certificate A); 677,607 experiments were carried out in which the animals were allowed to recover from the anaesthetic (certificate B), and only 171,283 experiments (3.7 per cent of the total) were carried out with anaesthesia for the whole experiment.

Many of the procedures carried out under certificate A must, by their very nature, cause great distress. For example in 1980, 484,849

animals were used in acute toxicity tests, of which the LD₅₀ is an example. In this case a recent government committee¹ conceded, "...LD₅₀s must cause appreciable pain to a proportion of the animals subjected to them". Most experiments involving application of substances to animals' eyes, and psychological experiments employing aversive stimuli, are two more examples of procedures carried out under certificate A.

The Council of Europe's discussion of article 8 of the draft convention is *not* concerned with permitting "licensed experiments causing pain without anaesthesia". Rather it is debating the presence of a pain clause which seeks to regulate the severity and duration of pain inflicted during the course of the experiment.

In Britain the pain condition directs that animals suffering pain which is "severe" or "likely to endure" should be painlessly killed "if the main result of the experiment has been achieved". Or if they are suffering "severe pain which is likely to endure", they should be painlessly killed.

Unfortunately no one has ever defined "severe" or "likely to endure". Indeed it is impossible to define "severe" since any assessment is largely subjective. (The LD₅₀ test is allowed to proceed for 14 days, unless death intervenes, but does this contravene the pain condition?) Here lies the central point — *pain cannot be regulated*. Realistically legislation can only license researchers, register premises and prohibit certain procedures. For example, in Great Britain licensees are not allowed to use animals to acquire manual dexterity.

The legislative way forward is therefore to prohibit those procedures which have become unacceptable in an evolving moral climate.

ROBERT SHARPE

Sheffield, UK

1. Report on the LD50 Test, Advisory Committee on the Administration of the Cruelty to Animals Act 1876 (Home Office, London 1979).

Anti-social?

SIR — In his address to the British Association¹, Sir Edmund Leach states that he encounters biologists who refer to "primitive" and "advanced" populations, and he goes on to say that these unfortunate individuals inevitably associate the advanced category with the group to which they belong. Later in his address Professor Leach refers to physical anthropology as a "tiny, rather off-beat sub-branch of the general field of anthropology" and that the "variety of human culture" (social anthropology) is "the main concern of anthropologists". Perhaps I am being "astonishingly naive" (an apparent failing of those interested in the trivia of human origins) if I ask whether it was merely coincidence that social anthropology is "the particular human collectivity to which the author himself happens to belong"?

Professor Leach proposes that Wilberforce was essentially correct in seeing an unbridgeable gap between man and ape. The basis for this assertion is the supposed uniqueness of human language. However, the fact that Leach, a social anthropologist,

should use such an important occasion as the 150th BA anniversary to put forward a model of the relationship of man to the primates testifies to physical anthropology being more than off-beat, and to the continuing primacy of Darwinian ways of thought. Is it likely that speculations based on miscellaneous observations of the variety of human culture will be able to solve the problem of how, why and when the divide between humans and apes occurred, let alone define the nature of it? Palaeoanthropology and various branches of biology are in the best position to test such a proposition, especially given that the foundation of human language is ultimately anatomical.

In the early 1970s Professor Leach launched an attack on the archaeologists² largely on the grounds that they were poor social anthropologists. Now it is the turn of the biological anthropologists, although this time the crime is that of not being social anthropologists at all.

ROBERT FOLEY

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University of Durham, Durham, UK

1. Leach, E.R. *Nature* 293, 19–21 (1981).

2. Leach, E.R. in *The Explanation of Culture Change* (ed. Refrew, C.) (Duckworth, London, 1973).

Incendiary subject

SIR — Perhaps you are right, after all, to describe Dr Rupert Sheldrake's *A New Science of Life* as a book for burning (*Nature* 24 September, p.245). For after seeing the disastrous effect Sheldrake's book has wrought upon the detachment, not to say the common sense, of one with the responsibilities of the editorship of *Nature*, I shudder to contemplate the effect upon the ordinary man.

But perhaps it is the influence of a pulpit from which to denounce scientific heresies that is the danger, rather than the book itself. For surely there is nothing in the book to raise excitement to the point of lumping together "creationists, anti-reductionists, neo-Lamarckians and the rest". For scientists, the worst a book can do is to waste their time. You could have served us better by arranging for the publication of two careful and opposed critical reviews. For non-scientists, unhelpful books abound. Their ability to mystify science is as nothing compared with strenuous attempts to declare an orthodoxy.

ROBERT HEDGES

Oxford University, UK

SIR — Before reading "A book for burning" in which Sheldrake's work *A New Science of Life* is criticized (*Nature* 24 September, p.245), I did not think I would have to ask a *Nature* leader writer to read or re-read Milton's *Areopagitica* — "as good almost kill a man as kill a good book. Who kills a man kills a reasonable creature, God's image; but he who destroys a good book, kills reason itself, kills the image of God, as it were in the eye. Many a man lives a burden to the earth; but a good book is the precious life-blood of a master spirit, embalmed and treasured up on purpose to a life beyond life".

Continued on page 594

NEWS AND VIEWS

Flightlessness and fear of flying in island species

from Jared M. Diamond

"I have heretofore asked the question concerning Mauritius henns and dodos, thatt seeing those could neither fly nor swymme, beeing cloven footed and withoutt wings on an island far from any other land, and none to bee seence elce where, how they shold come thither?" (from *The Travels of Peter Mundy in Europe and Asia 1608–1667*, ref. 1).

The traveller Peter Mundy, who in 1636 visited the remote island of Mauritius 2,000 km from the African coast, was surprised to find flightless birds there: the Dodo and the rail ('henn') *Aphanapteryx bonasia*. These two birds are part of a larger problem that has puzzled many biologists since. We now know that most remote islands do (or did until man's arrival) harbour flightless birds and insects. Other island birds, bats and insects are 'psychologically flightless': they refuse to fly across water, despite flying readily overland. Islands also harbour the equivalent of flightless plants: species whose fruits lack morphological adaptations, present in

fruits of related species, for overwater dispersal. There is an analogous phenomenon among shallow-water fish and molluscs. Biologists ask with Mundy, how should all these flightless creatures (*sensu latissimo*) come to occur on remote islands that never had continental connections?

A recent treatment by Feduccia², building on an earlier study by Olson³, brings us closer to understanding the problem for flightless birds. Feduccia and Olson are concerned with the questions: who? where? why? how? how fast?

Who are the flightless birds? The prize goes to the family Rallidae (rails, including coot, crakes and gallinules), which includes at least 53 extant or recent flightless species, over one-quarter of all the species in this family. Other flightless birds include (or included) the Dodo (derived from pigeons), ratites (ostrich, rheas, emus, cassowaries, and the extinct moas and elephant birds), and various species of duck, goose, parrot, cormorant, grebe, ibis

and owl. As for the question 'Where?', all but one of the known flightless rails are or were on islands, where many were quickly exterminated after humans arrived. Almost every remote island explored for bird fossils has yielded a recently extinct flightless rail. Of flightless birds other than rails, some are also on islands (New Caledonia, New Zealand, Madagascar, Hawaii, Jamaica), but continents harbour others (ostrich, rheas and the grebes of Lakes Titicaca and Atitlan).

"Why, and how, so many species of rails and other birds have on so many occasions so speedily renounced their heritage of millions of years of aerodynamic refinements is a subject of great interest" (Olson, ref. 3, p.31). As for the 'Why?', a few island flightless birds, such as New Zealand's moas and Madagascar's elephant birds, were enabled by flightlessness to do something impossible for a flying bird: they became giant grazers on islands lacking grazing mammals. What *permitted* flightlessness to evolve on islands is that wings are unnecessary there: no mammalian predators to flee, until man arrived with his commensal cats and rats to begin the work of extermination; and reduced need for dispersal on a small homogeneous island compared with the widely spaced habitat patches of continents. But it is not enough to show that flightlessness is permitted: why is it actually *favoured*? Biologists have often worried over this question in explaining the loss of other unnecessary structures, such as functional eyes in cave animals and electric eels. Olson and Feduccia illustrate dramatically the energetic advantages of flightlessness when wings become unnecessary. The muscles and bones that constitute the flying apparatus represent 20–25 per cent of the weight of typical birds. Furthermore, the specialized flight muscles of birds rank among the most expensive of all tissues in their energy consumption. The energy saved by flightlessness can be put to other purposes. A winged rail on a predator-free island is like a 60-kg back-

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From *Vanishing Birds* by T. Halliday (Penguin, 1981).

Jared M. Diamond is Professor of Physiology at the University of California Medical School, Los Angeles.

packer forced eternally to carry 15 kg of bricks and to regurgitate half of each meal.

Olson and Feduccia have also clarified 'How' flightlessness evolved. At first sight, it appears to involve many independent changes, such as reduction of the muscles and bones of the wing and pectoral girdle, reduction of the keel, and increase in angle between the scapula and coracoid. In fact, almost all birds are flightless as chicks, and the anatomical differences between chicks and adults of flying birds are quite similar to the anatomical differences between adult flightless and adult flying birds. Thus, flightlessness could arise through a change in only one or a few developmental genes, arresting perinatal development at an earlier stage. (Similarly, differences in a small number of developmental genes could explain why humans look and behave quite differently from chimpanzees and gorillas, despite our close similarity to apes in overall protein and DNA.) This insight suggests why rails are so prone to become flightless: the sternum begins to ossify only many weeks after hatching in rails, but before hatching in chickens. "It is this postponed development of the sternal apparatus that allows neoteny in rails to result in the loss of the flight apparatus without also resulting in the loss of the rail . . . For [chickens] to give rise to flightless species, development would have to be arrested far in advance of hatching, when other organs have not yet developed, and the results might well be lethal" (Feduccia, ref.2, p.111-112).

How fast can flightlessness evolve? For flightless birds with no close relatives, we have no idea. But some flightless birds are otherwise exceedingly similar to flying relatives, implying that evolution of flightlessness has been rapid and recent. For example, the duck *Anas aucklandica* has flying subspecies on New Zealand and Campbell Island and a flightless one on Auckland Island, and there is a similar example in the rail *Dryolimnas cuvieri*. The rails *Gallinula nesiotis* of Tristan and Gough Islands, *Fulica newtonii* of Mauritius and Reunion Islands, and *Fulica chathamensis* of New Zealand and the Chatham Islands, consist of very similar subspecies, both flightless, on two distant islands, both populations having evidently evolved flightlessness independently and recently from a common ancestor. Olson suspects that the actual time span to evolve flightlessness in rails is measured in generations, not millennia.

Islands as well as some continental areas also harbour flightless insects with flying relatives, such as every insect of sub-Antarctic Heard Island⁴, and a wingless fly and a flightless moth that jumps like a grasshopper on Campbell Island⁵. Some, but not all, of these flightless insects are on wind-swept islands or mountain tops where flight would doom an insect to being swept away. We know that evolution of flightlessness can be rapid in some insects: many corexids bug⁶ and waterstriders⁷ have

populations that shift between flight and flightlessness with change in season, temperature and food supply.

For plants the equivalents of flight are those adaptations of seeds permitting them to be carried overwater: parachutes favouring wind dispersal, barbs or glue that catch on birds' feathers, buoyancy and impermeability permitting transport by ocean currents, and light weight and a nutritious coat inviting consumption by birds. The seeds of numerous island plants have become figuratively flightless by losing these adaptations of the seeds of their ancestors⁵.

While the birds, insects and plants discussed above have achieved 'flightlessness' by morphological change, other animals have achieved it behaviourally, through fear of flying. Especially in tropical rainforest, hundreds of bird species that fly over land refuse to cross water gaps of even a few hundred metres (for example, see ref.8). The only islands on which such species occur are ones formerly connected to continents by land bridges, like the Pleistocene bridges that account for the presence of rhinoceroses and other non-volant mammals on Java and Borneo. Entire families of birds are afflicted with fear of flying overwater and that, like rhinoceroses, are virtually confined to land-bridge islands include the *Furnariidae*, *Formicariidae*, *Dendrocolaptidae* and *Rhamphastidae* in the neotropics and the *Eurylaimidae* and *Garrulidae* in the Oriental Region⁹. Some species thus restricted are powerful fliers overland, including hawks, swifts and parrots; hence there can be no doubt that the failure to cross narrow water gaps is through choice, not inability. Bats afflicted with fear of flying overwater include *Pteropus mahaganus*, *Pteralopex atrata* and *Anthops ornatus*, confined today to four

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large Solomon islands that were joined by Pleistocene land bridges¹⁰. Among butterflies there are clear behavioural differences between species that are, and are not, good overwater colonists: in strong winds the former continue to fly, the latter crouch within vegetation¹¹.

Finally, an underwater equivalent of flightlessness or fear of flying occurs among shallow-water fish and molluscs¹². At one extreme, some species of fish and molluscs of coral reefs have larvae that remain in the plankton for long times; such species tend to occur in genetically similar populations on reefs thousands of kilometres apart. At the other extreme are species whose larvae settle on a substrate within a day or that lack pelagic larvae and exhibit parental care of the young. Such species tend to be confined to single reef systems or to occur on different reefs in genetically distinct forms, because of poor dispersal over deep water.

The short message is: for diverse animals and plants, the anatomical and behavioural bases of dispersal are subject to natural selection and may differ enormously between species within the same genus, just as Olson and Feduccia have traced for birds. □

1. *The Travels of Peter Mundy in Europe and Asia 1608-1667* 5, 83 (Hakluyt Society, London, 1936).
2. Feduccia *The Age of Birds* Chapter 6 (Harvard University Press, 1980).
3. *Smithsonian Contr. Zool.* no.152 (1973).
4. Gressitt *Pac. Insect Monogr.* 23, 295 (1979).
5. Carlquist *Island Biogeography* (Columbia University Press, 1974).
6. Young *J. Anim. Ecol.* 34, 353 (1965).
7. Jarvinen & Vepsäläinen *Hereditas* 84, 61 (1976).
8. Willis *Ecol. Monogr.* 44, 153 (1974).
9. Terborgh in *Tropical Ecological Systems* (eds Golley & Medina) 369 (Springer, Berlin, 1975).
10. Phillips *Univ. Kansas Publ. Mus. Nat. Hist.* 16, 777 (1968).
11. Holloway *The Lepidoptera of Norfolk Island* (Junk, The Hague, 1977).
12. Kohn *Pac. Sci.* 15, 163 (1961); Ehrlich *A. Rev. Ecol. Syst.* 6, 211 (1975); Leis & Miller *Mar. Biol.* 36, 359 (1976).

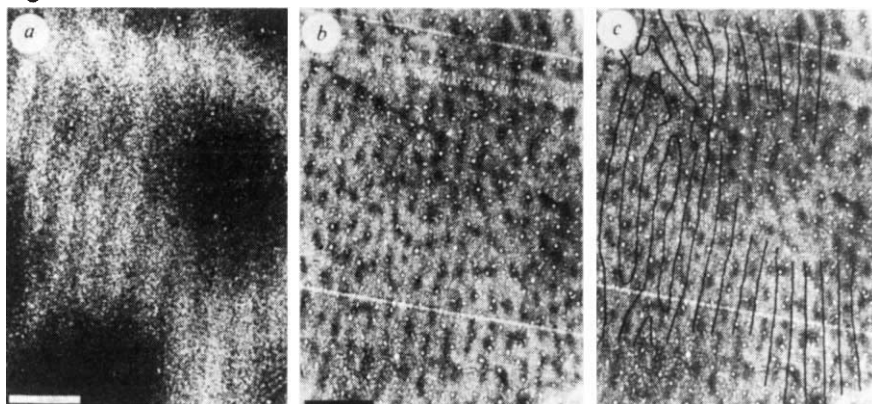
Patches in monkey visual cortex

from N. V. Swindale

IN RECENT YEARS the deoxyglucose method has played an important part in studies of columnar systems in the visual cortex, but until now, the evidence linking the patterns of glucose uptake to the patterns of physiological responsiveness observed by microelectrode recording has been indirect. The link has always been very strong however, and it comes as no surprise that a paper by Schoppmann and Stryker¹, published in this week's issue of *Nature* (p.574), should show, in cats stimulated with moving vertical bars, a strong correlation between regions of high deoxyglucose uptake and regions where cells respond best to vertical orientations. It is reassuring to have this result, nevertheless, because recent work on the monkey visual cortex by Hendrickson and her colleagues^{2,3} has questioned the assumption that local variation in the rate of glucose uptake within the visual cortex must necessarily be the result of a corresponding variation in neural firing rates.

The story begins with the demonstration by Hendrickson, Hunt and Wu³ that the visual cortex of macaque monkeys contains cell bodies and presynaptic processes that stain heavily for γ -amino decarboxylase (GAD), an enzyme required for the synthesis of γ -amino butyric acid (GABA). Similar findings have been made in the rat⁴ and this adds to the already good evidence that GABA is a transmitter in the visual cortex of these and other mammals. In the rat, GAD is evenly distributed throughout the cortical layers, but in the monkey there is some laminar variation in the intensity of staining, and intriguingly, an increased intensity of GAD staining in layers II and III in small round or oval patches that are about 100–200 μ m in diameter and spaced about 400 μ m apart.

Rows of patches are superimposed on the centre of either a left or a right eye ocular dominance column, and in monkeys whose visual experience was normal up to the time of death, each patch is a region of high metabolic activity. This last conclusion is based on the use of a stain for the mitochondrial enzyme, cytochrome oxidase. Like the deoxyglucose technique, this stain can be used to reveal local variations in metabolic activity^{5,6}, the difference between the two methods being that levels of cytochrome oxidase change much more slowly than the rate of glucose uptake in response to alterations in metabolic demand. Sections of tissue stained for cytochrome oxidase show patches of high activity in layers II and III, and the patches are aligned both with the ocular dominance columns^{3,7} and with the



a, An autoradiograph of striate cortex from a monkey injected with tritiated proline in one eye. Columns dominated by the injected eye are seen as light bands of label. **b**, Cytochrome oxidase staining, which reflects increased metabolic activity, reveals rows of patches in a more superficial section from the same block as **a**. **c**, The rows of cytochrome oxidase patches can be seen to follow the pattern of ocular dominance patches by drawing the borders of the columns from **a** directly onto **b**. Deoxyglucose labelling after stimulating one eye with stripes in all orientations reveals patches that lie in register with alternate rows of cytochrome oxidase patches. From ref. 7.

regions where the GAD staining is most intense.

Hendrickson *et al.*³ interpret these results to mean that the energy requirements of nerve cells vary significantly according to transmitter type. Since these cell types may be inhomogeneously distributed, this may significantly complicate the interpretation of deoxyglucose experiments. In support of this idea it has been shown that deoxyglucose autoradiographs from either normal monkeys⁷ or monkeys stimulated with moving bars of all orientations and varying widths and velocities² (the intention being to stimulate all cortical neurones equally) show patches of high glucose uptake in layers II and III which coincide with the positions of the cytochrome oxidase patches. The results of Schoppmann and Stryker from the cat are not actually in conflict with this, for normal cats lack the patchy distribution of cytochrome oxidase⁷ and, unlike the monkey, the control condition of stimulation with all orientations results in a uniform distribution of glucose uptake within any one layer¹.

An alternative and more conventional explanation for the deoxyglucose and cytochrome oxidase results in the monkey can easily be found, however. It might be that with normal visual experience, cells within the patches are active more often than cells outside them, either because they were more responsive than other cells, or because they responded equally well to edges of all orientations. The increase in cytochrome oxidase levels might be a secondary consequence of this since several experiments^{6,7} have shown that cytochrome oxidase levels change in response to long-term alterations in electrical activity. Horton and Hubel⁷ give a nice demonstration of this: if one eye of an adult monkey is removed, then 10 days

later the cytochrome oxidase patches aligned on that eye's ocular dominance stripes become pale and shrunken, giving a pattern of alternating rows of large and small patches. The raised GAD levels might also be a secondary consequence of increased impulse activity in the patches since it is possible that the patches do not contain greater numbers of GAD-positive cells, but simply cells containing a greater density of GAD. Repetition of Horton and Hubel's experiment using GAD staining to reveal the patches would be a good test of this possibility.

The existence of groups of non-orientation selective cells in layers II and III is also consistent with the results of a second experiment performed by Horton and Hubel. This showed that in monkeys stimulated with either horizontal or vertical stripes, there were always regions of high deoxyglucose uptake that coincided with the positions of the cytochrome oxidase patches (because the period of selective stimulation was short in these experiments, it can be assumed that cytochrome oxidase levels had not changed from their prior state). In the words of the authors, "all orientations of line stimuli probably activate cortex in the regions of the patches". Regions of high deoxyglucose uptake outside the patches presumably contain cells selective for the orientation of the visual stimulus, and since these regions are continuous with the patches,

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irrespective of the orientation used, the results imply that different iso-orientation domains meet in, or near, the patches. If true, this is a fascinating result, for it suggests a structural link between orientation and ocular dominance columns of an unexpected and subtle kind. Previous work⁸ had suggested no obvious relationship — for example the two patterns have different periodicities, and the angles at which the columns intersect

locally are apparently random.

It is likely that the uncertainties of interpretation raised by these experiments will be resolved soon. Experiments are in progress to determine the quantitative relationship between neural discharge frequency and rate of glucose uptake⁹, and the preliminary results suggest that even a small increase in maintained discharge would cause a significant increase in glucose uptake. It should be straight-

forward to discover whether the firing rates of cells in the patches are normally higher, on average, than those of cells elsewhere in layers II and III and, if so, whether it is because the cells lack orientation specificity, or for some other reason. It would also be useful to have an estimate, even a crude one, of the relative amounts of energy devoted by nerve cells to the generation of action potentials and to the synthesis of transmitters such as GABA. □

Building a nervous system

from Hilary J. Anderson

RECENT studies of the development of the grasshopper nervous system are beginning to suggest some of the strategies that might be used to generate complex neural networks from simple basic structures. The studies have been made possible by the special advantages offered by grasshopper embryos. They are sufficiently transparent that, with the aid of interference contrast optics, individual neuron precursors can be identified and watched as they divide and give birth to neurones. The precise lineage of a particular cell may thus be determined and used as to identify the cell before it has developed any outward signs of differentiation. The embryonic nervous system is also in a superficial and accessible position. Neurones may thus be readily impaled with recording microelectrodes or filled with dyes to obtain information about their electrophysiological and morphological development.

Two key points have emerged from following the developmental fate of individual neurons. First, certain neurons play a distinctive 'pioneering' role in establishing the basic plan of tracts within the CNS at an early stage when axons need cover only small distances by simple direct routes to find their targets. Second, although the segmental ganglia of the nervous system are each distinctly different in the adult, they are formed from identical sets of precursor cells. Some differences between ganglia can be generated at early stages by variation in the extent to which developing neurons survive and differentiate. These small differences may then lead, through a cascade of effects mediated by cellular interactions, to the complex diversity seen in the mature ganglia.

Segmental organization

The central nervous system of the grasshopper consists of a chain of segmental ganglia each of which supplies nerves to one segment of the body (see figure 1). The ganglia differ in their structure and are specialized for different behavioural functions. For example, the three thoracic ganglia have about 3,000 neurones each and supply nerves to the legs and wings and contain the basic circuitry for generating the patterned output for

flying and walking. Most of the eleven abdominal body segments bear no appendages and their ganglia have only some 500 neurones each.

In view of these differences it is remarkable that each ganglion arises from an identical set of precursor cells^{1,2}. The precursors appear when the embryo consists only of a sheet of ectoderm overlain by a layer of mesoderm. Two types of precursor have been identified within the ectoderm: neuroblasts^{1,3}, and midline precursors cells², and they are always arranged in the same characteristic pattern as in figure 1.

In each segment there is a fixed stem cell population of 61 neuroblasts which divide repeatedly to bud off daughters which themselves divide to produce neurones. Each neuroblast produces 10-100 neurones before dying. The midline precursor cells

have not been described in any other nervous system. There are seven in each segment and they undergo only one division to produce a pair of daughters which become neurones.

Constancy of neuron differentiation

Electrophysiological and morphological studies of individual neurons have shown that a neuron with a particular lineage from a particular neuroblast or midline precursor always develops into the same adult neuron, and acquires its particular properties in a precise temporal sequence⁴⁻⁷ (see the table).

Establishment of neuronal tracts

Each adult ganglion has an outer layer of neuron cell bodies surrounding a dense core of neuron processes where synapses occur. Through this core runs a maze of tracts forming long-distance axon pathways between neurons in different parts of

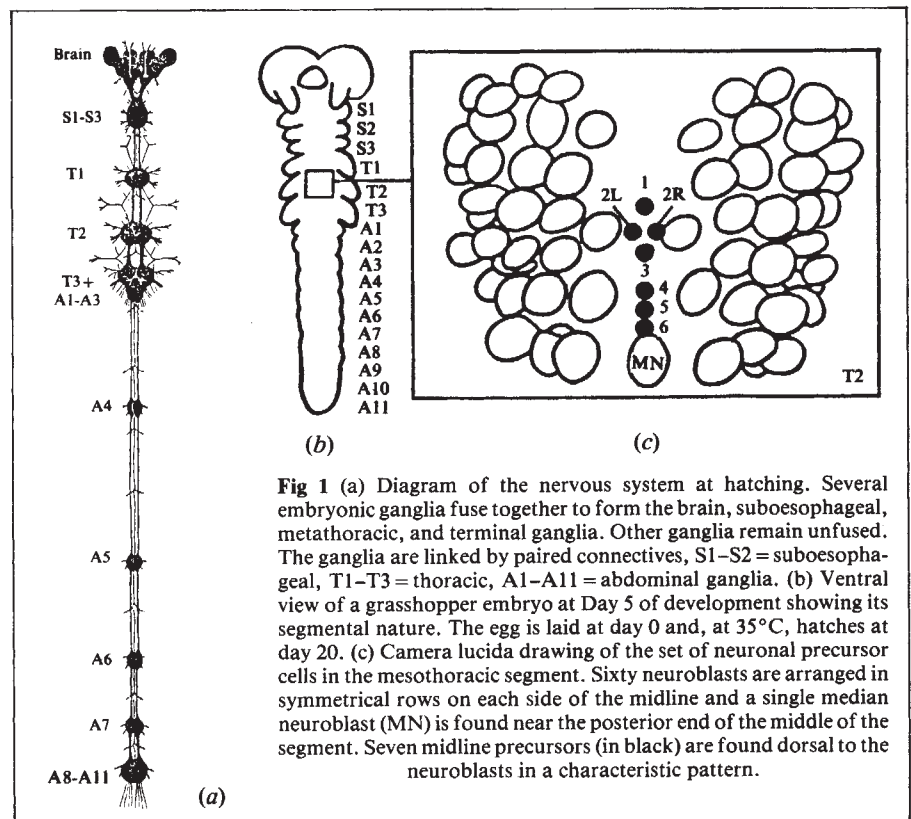


Fig 1 (a) Diagram of the nervous system at hatching. Several embryonic ganglia fuse together to form the brain, subesophageal, metathoracic, and terminal ganglia. Other ganglia remain unfused. The ganglia are linked by paired connectives, S1-S2 = subesophageal, T1-T3 = thoracic, A1-A11 = abdominal ganglia. (b) Ventral view of a grasshopper embryo at Day 5 of development showing its segmental nature. The egg is laid at day 0 and, at 35°C, hatches at day 20. (c) Camera lucida drawing of the set of neuronal precursor cells in the mesothoracic segment. Sixty neuroblasts are arranged in symmetrical rows on each side of the midline and a single median neuroblast (MN) is found near the posterior end of the middle of the segment. Seven midline precursors (in black) are found dorsal to the neuroblasts in a characteristic pattern.

the nervous system. The initial framework for this complex system of tracts is very simple and is laid down within each ganglion at early embryonic stages. The framework is a rectangular pattern of nerves running transversely to link right and left halves of each ganglion and longitudinally to link each ganglion with its neighbours² (see figure 2). As development proceeds other simple pathways are added⁷. Some of the neurons which form these pathways have been identified and found to be the same in every segment^{2,7}. They have been termed 'pioneer' neurons and are amongst the very first neurons to differentiate and produce axons. New neurons develop their axons and grow along the pioneer pathways^{2,7} much more quickly than pioneering axon growth. At points where two or more pathways cross, the growing axon tips extend many fine branches in all directions. Later all these branches are withdrawn except for those growing along the correct pathway⁴.

Variations on a segmental theme

How do the same sets of precursor cells give rise to ganglia which are different? One clue is the vast differences in the number of cells that die in different developing ganglia. Closer inspection

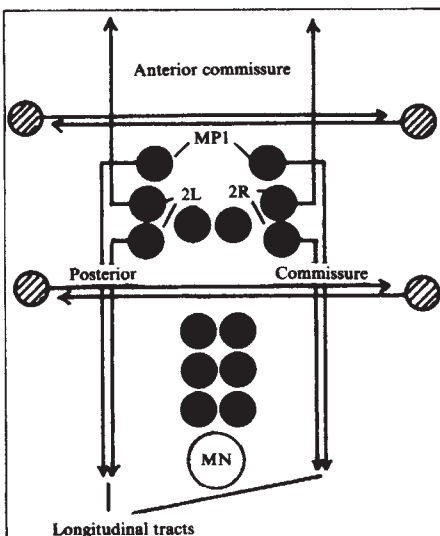


Fig 2 Diagram of the rectangular pattern of pioneering pathways laid down in each segment by the axons of neurons produced by divisions of three of the midline precursors (MP1, MP2L, MP2R) and some unidentified lateral neuroblasts (cross-hatched).

reveals that both neuroblasts and neurons die with several effects on the shaping of the ganglia. First, the number of neurons produced in each ganglion is regulated by the lifespan of its neuroblasts; these die much earlier in the abdominal ganglia and so produce many fewer neurons¹. Second, large numbers of neurons die, many after they have differentiated and produced axons^{1,8}. This also affects neuron number and thus ganglion size.

However, cell death has other important consequences. The loss of different

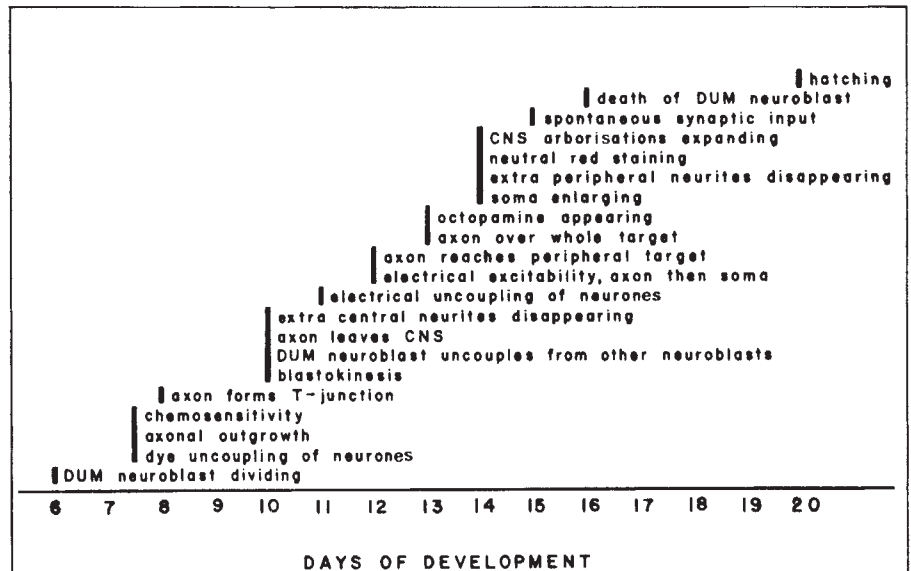


Table 1 Timetable of neuron differentiation at 35°C, as exemplified by DUMETi, one of the median neuroblast progeny⁴. Morphological development is characterised by an initial profusion of growing neurites and axonal branches, some even extending down the "incorrect" peripheral nerves. Supernumerary neurites start to disappear as the axons leave the CNS, but extra axonal branches are not lost until the target muscle is reached. Then the central arborizations begin to develop and the soma enlarges to its full size. Physiological development is characterised by a precise sequence of events including the loss of electrical and dye coupling with other cells, and the appearance of chemosensitivity, electrical excitability, and neurotransmitter.

populations of neurons in different ganglia produces dramatic differences in the spatial arrangements of those remaining — simple axon pathways become shifted into more complex patterns, some neurons lose their neighbours while other distant neurons become neighbours. Since communication between cells is important for many developmental decisions, it is likely that the alteration of neighbour relationships results in altered patterns of cell communication and this generates further diversity among the neurons of different ganglia.

Indirect evidence for this view has been obtained by studying homologous neurons in different ganglia (that is, neurons produced by the same lineage from equivalent neuroblasts or midline precursors). Homologues indeed differ in their survival rate and also in the final differentiated form which they attain^{8,9}. For example, in the thoracic ganglia one of the daughters of midline precursor 3 (see fig 1c) develops into a fully mature neuron with particular electrophysiological properties and a characteristic morphology. In more posterior ganglia there is a progressive reduction in the extent to which the homologues of this neuron differentiate. In the third abdominal ganglion, for example, the homologous neuron has a simplified morphology and only its axon becomes electrically excitable, while in the fourth abdominal ganglion the homologue dies⁹. Another example is that of neurons which, in the thorax, innervate the thoracic leg muscles. There are no legs in the abdomen and the homologous neurons produced in the abdominal ganglia die shortly after forming their axons. Recent

experiments have shown that the cause of this cell death is not the lack of appropriate target muscles⁸. Whether it results from some other subtle cellular interaction or from specific differences in commitment that homologous neurons in different segments acquire by lineage remains to be clarified⁸.

In conclusion it may be said that there has been remarkable progress since 1976 when Bate wrote that "the embryogenesis of the insect nervous system remains an almost completely uncharted subject" (see ref. 1). The detailed descriptions obtained in the last five years have established that there are simple origins to the complex adult structure. This now permits the question "How is the structure of the insect CNS established?" to be rephrased at a new level of resolution: "How do pioneer neurons navigate through the embryonic nervous system?" or "How do growing neurons recognise appropriate pathways?" or "What are the mechanisms controlling the death of neuroblasts and neurons?" □

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Inside a volcano: the first three-dimensional map

from R.S.J. Sparks

KILAUEA VOLCANO, HAWAII, has long been regarded as about the most thoroughly documented and best understood active volcano on Earth. Since 1952, increasingly sophisticated instruments have been used at the Hawaiian Volcanological Observatory to record the ground deformation caused by swelling of the volcano before major eruptions and the seismicity brought about by basalt magma moving beneath the volcano. Every eruption has been carefully documented with the volumes, discharge rates and lava composition measured on an almost daily basis during periods of activity.

The research has culminated in a recent paper by a team of US Geological Survey scientists (Ryan, Koyanagi & Fiske *J. geophys. Res.* **86**; 7111, 1981), in which an analysis of all the geophysical and volcanological data from 1969 to 1974 is used to construct a three-dimensional model of the internal structure of Kilauea's magmatic reservoir and transport system. The period was a particularly eventful one with several eruptions at the summit and the formation of the Mauna Ulu shield volcano on the east rift zone.

A clear Plexiglass scale-model containing 18 depth levels at 1 km spacing was constructed, upon which well located seismic hypocentres were plotted. The seismic network at Kilauea enables most earthquake sources to be located to better than 600 m in both horizontal and vertical coordinates, and it is possible to distinguish between earthquakes induced by fracturing of rock by magma under high fluid pressure and those produced by dry cracking of rock. When the magmatic events are plotted and placed in the three-dimensional model, the zones of active magma transport can be identified down to a depth of 18 km. This is the first time that the three-dimensional structure of an active magma reservoir system has been defined.

The model reveals some fascinating features of the Kilauea plumbing system. A well defined central magma storage system or chamber is located beneath Kilauea summit caldera from 2 to 6 km depth. The top of the magma chamber is defined by an elliptical zone of seismic activity at the 1.1 to 2.6 km levels. The interval from 5.7 to 6.5 km depth is nearly aseismic and is believed to represent the floor of the storage chamber. Modelling of the ground deformation of the volcano also confirms that the magma reservoir can be treated as a source of pressure at depths in the range of 2.5 to 4 km (Dieterich & Decker *J. geophys. Res.* **80**; 4094, 1975). The reservoir has a capacity of between 5 and 10 km³ of basalt magma and is envisaged as a plexus of

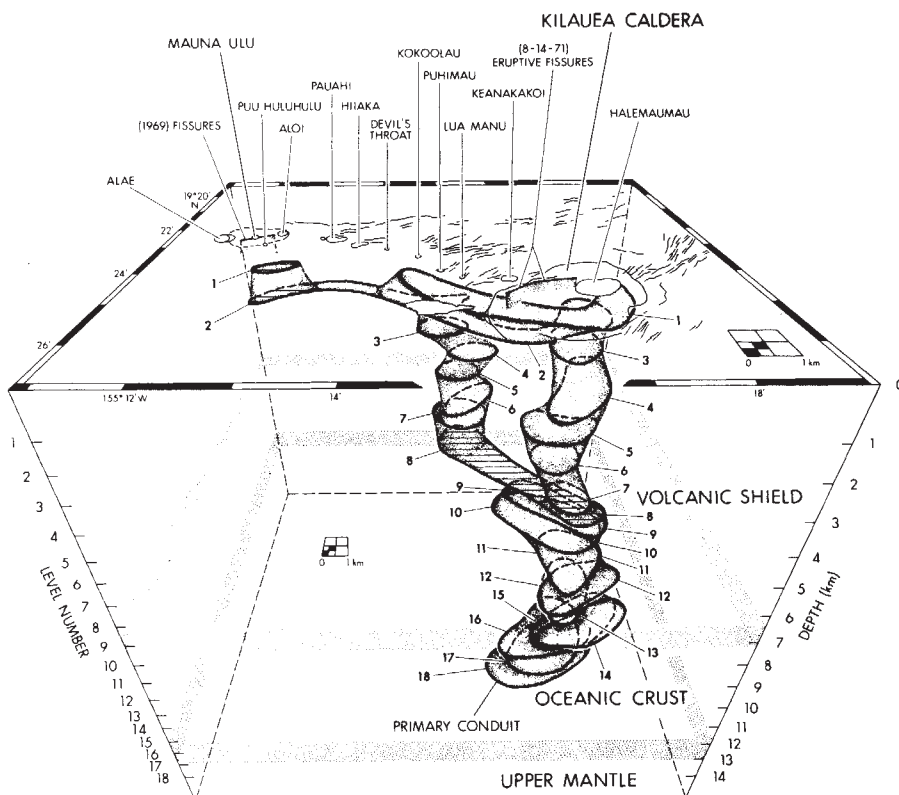
interconnected intrusions.

A primary conduit feeds magma from the upper mantle at 14.6 km into the base of the shallow-level storage system. The conduit has been defined by seismic events that cluster tightly at different levels, forming a columnar region with a vertical axis and elliptical horizontal cross-sections. It is important to appreciate that this conduit cannot be a totally magma-filled pipe, but rather consists of "honeycombed magma-filled fracture zones with appreciable fluid-to-rock ratios", that is, a zone through which magma migrates along fractures to the summit reservoir. Over the past 30 years basalt magma has been transported through this system at a remarkable steady rate of 3 m³ s⁻¹.

A second shallow-level pipe in the upper part of the east rift zone has also been identified. The zone has been the site of many eruptions and a close connection has been established between deflation of the ground above the summit reservoir and flank eruptions on the rift. Magma can be fed from the base of the main reservoir up this secondary pipe to the east rift and, in addition, earthquakes define an intense zone of intrusion, named the east rift zone duct, which feeds magma down the east rift at depths of less than 2.0 km. A dyke swarm is probably forming in this zone.

The new model of the plumbing system is of great importance in understanding the origin of the basaltic lavas of Hawaii. The detailed petrology and geochemistry of the lavas documented for every eruption since 1952 can now be tied in with a well constrained model of the reservoir and transport system. The variations in temperature and composition can be investigated with a good idea of where each batch of magma originates. For example, there is a general tendency for the east rift zone magma fed from the summit to be hotter than that erupted at the summit, consistent with diversion of hotter magma from the base of the summit reservoir or directly from the primary conduit below into the east rift zone pipe.

The modelling technique may also prove valuable in more productive monitoring and prediction of the volcano's behaviour. As more data are made available, the movement of magma within the volcano and changes in the reservoir and transport system can be observed. The study shows that there is no substitute for the continuous and year-by-year documentation of an active volcano. It is not just the authors of this paper who are to be congratulated for the success of the Hawaiian monitoring programme, but the many volcanologists working on Hawaii over more than 20 years. □



Southward view, looking down into the interior of Kilauea. The transition zones from volcanic shield to oceanic crust, and from oceanic crust to upper mantle are stippled. Square, 1 km scale grids are at the 0.0 and 14.7 km depth levels.

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Red giants and their descendants —new light on old stars

from Ben Zuckerman

RED giant and supergiant stars have long been favourites of professional and amateur astronomers. These big, bright stars emit up to 100,000 times more light than our Sun and so are easy to study. Some of them, specifically the pulsating long-period variables, significantly change their size, brightness and colour within about a year, a time scale of interest to a single human being. Recently IR and microwave astronomers have shed new light on the red giants and their descendants, the planetary nebulae.

The red giants occupy a crucial place in stellar evolution. When the core of a giant star exhausts its nuclear fuel and surpasses 1.4 times the present mass of our Sun ($M_{\odot}$), it is unable to support its own weight and collapses into a tiny neutron star. The gravitational energy released during this implosion of the core blows off the remainder of the star in a gigantic explosion — a supernova. Since around 50 per cent of all stars are believed to begin their lives with masses greater than $1.4 M_{\odot}$, we might expect that one out of every two stars will die as a supernova. But, in fact, only one star in about 30 dies such a violent death. The rest expire much more peacefully as planetary nebulae (see Fig.1). Apparently most massive stars manage to lose sufficient material before they exhaust their nuclear fuel so that their masses drop below the critical value of $1.4 M_{\odot}$. Most of this mass loss takes place while the star is a red giant.

As material flows out of a red giant star, it cools and dust grains form. The dust



Fig.1 A planetary nebula. The last remnant of the fluffy envelope of a former red giant is heated and ionized by the very hot central star which is the erstwhile core of the red giant. When the central star cools off, it will become first a white dwarf and then a black dwarf about the size of the Earth. (Palomar Observatory, California Institute of Technology photograph with the 5-m telescope.)

scatters and absorbs visual light emerging from the photosphere which it changes into IR radiation. If the mass outflow rate is large enough, so much dust condenses in the circumstellar cloud that it forms a cocoon that completely blocks the visible radiation from the star, so that only IR and radio radiation can escape. Indeed, IRC + 10216, a pulsating giant located 700 light years from the Earth, is the brightest source outside the Solar System in the entire $5 \mu\text{m}$ sky — it is the Sirius of the IR world — although visually it is fainter than 18th magnitude and thus difficult to see even with very large telescopes.

A huge rate of mass loss of $10^{-4} M_{\odot}$ per year has been deduced from IR observations of ammonia (NH_3) molecules located in the circumstellar cloud around IRC + 10216. Recent microwave observations of carbon monoxide (CO) molecules by M. Morris, M. Jura and A. Stark indicate a similar rate of mass loss and demonstrate that the escaping material extends outwards from the star for at least a light year and, possibly, a good deal further. This gigantic cloud completely dwarfs even the large photospheric diameter of stars such as Betelgeuse, the 0 magnitude red supergiant in Orion.

Because we know the size of the cloud around IRC + 10216 and can use either NH_3 or CO to measure the outflow velocity (15 km s^{-1}), we can calculate an age ($\sim 10,000$ years) for the circumstellar cloud. IRC + 10216 has apparently expelled, in the form of molecules and dust grains, a mass equal to that of our entire Sun within the past 10,000 years! This implies that some stars can shed huge amounts of matter very quickly and thus may never supernova. Statistics of supernovae and planetary nebulae as well as theoretical models suggest that stars which begin their lives with masses around $6 M_{\odot}$ (plus or minus a few solar masses), shed sufficient material to drop below the critical value of $1.4 M_{\odot}$. A star like IRC + 10216 could do this in a mere 50,000 years which is but an instant in the life cycle of all stars.

Thanks to an IR rocket survey carried out at the Air Force Geophysics Laboratory a few years ago, we now know that IRC + 10216 is not a freak, but simply the closest example of a class of very cool, pulsating star embedded in dense cocoons of dust and gas of their own making. IRC + 10216 is sufficiently close to have showed up in a ground-based Caltech survey at $2 \mu\text{m}$ wavelength carried out in the 1960s, but other cooler and/or more distant stars were too faint to be detected. Their discovery required the 4, 11 and $20 \mu\text{m}$ detectors on the AFGL rockets.

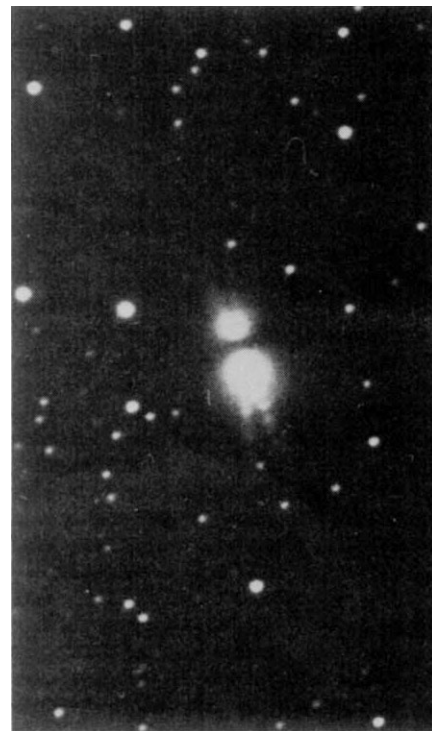


Fig.2 Photograph of the Egg Nebula taken by George Herbig using the Lick Observatory 120 inch telescope in blue light. Many molecules have been identified in the carbon-rich outflowing material around this and other proto-planetary nebulae. Some are common substances, such as methane and ammonia; others are exotic molecules never seen on the Earth.

One appealing interpretation of these IR giant stars is that they are 'proto-planetary nebulae' — old giant stars whose dense cores have almost but not quite rid themselves of the fluffy envelopes surrounding them. Once the star has lost the entire envelope, its exposed core becomes the central star of the planetary nebula which heats and ionizes the last vestiges of the envelope as it flows away into space. This is, of course, a fully fledged planetary nebula (Fig.1), long familiar to optical astronomers.

It is the stage just before this that has eluded optical astronomers for so long, simply because little or no visual light can penetrate the circumstellar cocoon. The AFGL rocket survey has, at last, yielded a number of excellent candidates for proto-planetary nebulae. The most famous is GL2688, dubbed the Egg Nebula by E. Ney. (V. Trimble has pointed out that GL2688 bears a much closer resemblance to a cat (see Fig.2) than to an egg!) In the model of GL2688 developed by Ney and his collaborators, the central star has expelled and is surrounded by an opaque torus (donut) of gas and dust that completely blocks the visual light and converts it to IR. So, GL2688 itself is a very intense and very

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cool IR source. However, light can escape through the holes of the donut and there it strikes additional material, presumably also ejected by the central star, which bounces part of the light in our direction. Thus we see two beautiful reflection nebulae in the shape of a cat.

Schmidt and Cohen (*Astrophys. J.* **246**; **444**, 1981) have made detailed spectro-polarimetric and spectrophotometric studies of two other bipolar nebulae that resemble GL2688. Dense condensations, and neutral gas in their shadows, are found to fill much of the volume of the nebulae, reinforcing the view that these bipolar objects represent a very early phase in the life of a planetary nebula. Recent models for these bipolar nebulae include biconical outflow above a luminous disk (Icke *Astrophys. J.* **247**; **152**, 1981) and gravitational ejection of matter from the atmosphere of a red giant star by a close companion star orbiting around it (Morris *Astrophys. J.*, in the press).

Radio astronomers have also studied GL2688 and find that substantial amounts of material probably exist well beyond the boundaries of the reflection nebulosities. Indeed, the total mass in the cloud around GL2688 may equal that around IRC+10216. More importantly, the clouds around both GL2688 and IRC+10216 are very rich in carbon; they are what we call carbon stars — relatively rare stars containing more carbon than oxygen rather than, as is typical, the reverse. More generally, most proto-planetary nebulae and, by extrapolation, planetary nebulae too are apparently carbon rich. These stars burn helium into carbon in their interiors and then expel the carbon into interstellar space, some day to be incorporated into new stars, planets and people. So much of the carbon in your scrambled eggs may have been cooked deep within objects like the Egg Nebula that lived, and died, many billions of years ago.

Where is this field heading now? Large new telescopes for IR and radio astronomy promise great advances and possibly some surprises in the ground-based study of cocoons around dying stars. But some of the most exciting discoveries should come after 1982 when NASA's infrared astronomical satellite is scheduled to be launched. The IRAS satellite will permit all-sky IR surveys at sensitivity levels much superior to all previous ground-based and rocket surveys. In addition to discovering more dying stars, IRAS should reveal numerous stars in the process of formation and even, perhaps, the IR radiation from a nearby super-advanced technical civilization (a Dyson sphere). □

Addendum

The meeting report "Extragalactic jets: facts and fancies" (*Nature* **293**, 336-337; 1981) was co-authored by Peter Scheuer and Mitchell Begelman. We apologize to Dr Begelman for omitting his name. □

Radiative frustration by vacuum manipulation

from Peter Knight

TRANSITIONS between atomic states are induced by incident electromagnetic fields. Even in the absence of an incident field, the vacuum fluctuations and zero-point energy of the quantized field interact with atoms and are responsible for spontaneous emission and for small radiative corrections to atomic energy levels. Spontaneous emission can be viewed crudely as stimulated by the ground-state quantum fluctuations in the electromagnetic field coupled to the atom. Ordinary stimulated transitions can be altered by changing the incident field frequency or intensity, but spontaneous decay would appear to be unalterable unless the field vacuum state can be changed. Recently Kleppner of MIT proposed¹ a method for doing just this, by placing the excited atom in a small high- Q cavity which 'squeezes out' some of the radiation modes and changes the mode density. The cavity can either frustrate or enhance the decay by a factor of the order of Q of the cavity, depending on the cavity detuning from atomic resonance². The effect of mode confinement on decay lifetimes provides new and direct information on the nature of spontaneous decay.

The idea of altering the radiation field vacuum state is an old one, going back at least to work in the 1940s on quantum Casimir forces between conducting plates. An enclosing cavity alters the boundary conditions of the problem and changes the appropriate mode expansion for the field. An elementary example of this is a one-dimensional cavity supporting only standing waves: an excited atom in such a cavity will have a strongly position-dependent lifetime which is enhanced or diminished depending in part on whether the atom is sited at a node or antinode of the field mode. In the 1960s and 1970s, Barton, Power and others used appropriate mode expansions and counterterms to uncover cavity-mode alterations in atomic energies, radiative corrections and lifetimes. This extensive theoretical work on energy shifts has been supplemented by meagre and rather negative experimental activity (see, for example, ref. 3).

Kleppner's suggestion, based in part on his Brandeis Summer Institute lectures of 1969, is to 'freeze out' vacuum modes by using a waveguide cavity with characteristic dimensions small compared with the decay radiation wavelength. This seemed to be unattainable with optical frequencies involved in normal decay. The alteration of spontaneous decay rates by dielectric wave-

guide structures had already been discussed by Wittke⁴, with particular emphasis on its importance in heterojunction light-emitting diodes; Kleppner has re-investigated waveguide effects but on highly excited Rydberg atoms. Such highly excited states decay slowly if they possess both a large principal quantum number and a large angular momentum; when they decay they do so slowly to a nearby Rydberg state, with the emission of very long-wavelength radiation. This enables larger and perhaps experimentally feasible waveguides to be used and Kleppner states that experiments are in progress.

More theoretical work will be needed before any experimental results can be properly evaluated. Kleppner concentrates solely on the 'mode density' and arrives at position-independent lifetimes from a modified golden rule. The atomic lifetime will clearly depend on the distance of the atom from the cavity wall simply because the 'inducing' vacuum field modes have a strong spatial dependence which is reflected in matrix elements; this has been omitted by Kleppner. His results seem to be an averaged-out approximation to the proper mode-expansion results and this needs examination. In particularly simple geometries (such as a pair of infinite mirrors separated by L , with an atom at position Z from one of the mirrors), the cavity-dependent lifetimes can be computed using proper mode expansions⁵ or image techniques⁶. The effect of cavity wall conductivity has been investigated in such simple systems using various models (see, for example, refs 7 and 8).

It might be thought that experiments on these effects using optical decay wavelengths would be extraordinarily difficult, this is not the case: using the Langmuir-Blodgett monomolecular layer technique it is possible to situate fluorescent molecules a fixed and microscopic distance between dielectric or metallic surfaces and to study the alteration of spontaneous lifetimes by an altered local environment⁹. Such experiments have revealed that spontaneous decay is not an immutable effect but is sensitive to the details of the confining geometry, and Kleppner's work indicates fascinating modifications to the properties of highly excited atoms in optical waveguides. □

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A *Nature* survey of the neurosciences

In one sense, the neurosciences are at the cutting edge of the new biology. Informed by new understanding and empowered with a staggering array of new techniques, they are engaged with one of the most teasing — and oldest — questions — “How does the physical representation of the mind, which is called the brain, carry out its functions?”. But in another sense, the neurosciences include some of the most tedious of investigations. The continual improvement of technique, staggering though it may be, is as often a source of frustration and disappointment as of discovery. This year’s new way with neurones could be next year’s discard.

Single experiments, such as investigations of the behavioural consequences of supposedly neurone-specific drugs or the consequences for neuronal development of particular forms of sensory deprivation, may take weeks or months. Often, the behaviour of neurones in, say, the human central nervous system must be inferred from that of model systems. (There are subliminal as well as formal inhibitions of the use of human brains.) In general, the neurosciences are in the business of making bricks without straw¹.

A further difficulty is the huge gap between the microscopic and the macroscopic. In an organ such as the liver (admittedly at one extreme), the biochemistry of a single cell is a good approximation to that of the whole. In the nervous system, however, cells with outwardly indistinguishable properties must in reality have different functions. The neurosciences are thus a challenge for the reductionists: can it be respectable to account for the properties of an integrated nervous system by giving a catalogue of neurones with different properties? The outward similarities of cells from nervous tissue have prompted an often exciting hunt for differences between them; the differentiation of a supposedly common cell type in such subtly different ways is, for the time being, neglected.

So why do the neurosciences remain buoyant? Part of the explanation is that the frustrations of laboratory work and of concept formation are more than outweighed by the underlying challenge. How, after all, does the brain work? Nobody knows, but many believe, as their predecessors have always thought, that they are within an ace of knowing.

What follows is an account, necessarily subjective, of where the neurosciences stand in the opinion of outside observers. It has been compiled partly by the reading of the journals (Nature not dominant but not negligible among them) but mostly by conversation.

The objective is not so much to startle neurobiologists themselves as to interest those who work in other fields in what their fellow-scientists are up to. For one of the abiding problems of the neurosciences is that they are poorly understood.

The techniques usually demand exceptional skill. The jargon is more than usually impenetrable because it has grown up by accretion from several disciplines — and with plenty of etymological licence: thus while a chemical that offsets the effects of some drug may properly be called its antagonist, why should another chemical with similar effects be called an agonist when no such word appears in English dictionaries (and the original Greek has a different meaning)? Then, like the spectroscopists of the early 1920s, the neurobiologists often give the impression of grubbing for data while lacking the leisure to consider the grand questions — what is it all for? and so on. The prizes will go, no doubt, to those who ask these questions.

Indeed, when asked what their long-term objectives are, most people say that philosophical speculations are the prerogative of retired persons, many of whom have been known to write books on the subject in their old age. Could it be that the neurosciences as they are would profit from a greater reflectiveness among their practitioners? Is it possible that in this field, as in high-energy physics a few years ago, something that might be called truth is obscured by too much data? Or does that opportunity still lie some way ahead?

Nobody can tell. Since the beginning, say in Galvani’s time, the most serious impediment to the neurosciences has been the difficulty of forming concepts. The recent influx of people from computer science — some of whom like to say they work in artificial intelligence — has helped a little, but not decisively. Maybe the outstanding problem of the neurosciences is to specify the problem to be solved.

Changing fashions can make sense

COLLECTIVELY, neurobiologists tend to give the impression of ebullience. Individually, they are defensive. The most provoking question is that of why, in the neurosciences, fashions come and go so quickly.

Why are the putative peptide neurotransmitters, widely thought to be important keys to understanding only three years ago, now more often canvassed as modulators of neuronal function? Why has the cleverness of those who are able to make intracellular recordings of the electrical behaviour of single neurones led chiefly to a recognition that the urgent need is for an understanding of how pairs of neurones function? Whatever happened, anyway, to the belief that memory resides in persistent chemical changes within nerve cells? (That, as it happens, is alive and well, but altered — see page 525.)

Trends

The defensive, sometimes tetchy but also justifiable response is that neurobiology is more than merely fashionable. "Neurobiology", the practitioners say, "has never been more exciting." And even last year's fashions, they insist, have served a useful purpose. This or that technique has yielded its harvest of data, its modicum of understanding. So who can say that this year's fashionable techniques may not bring home the bacon?

So what is now in vogue? One solid foundation for the future is emerging. Neurones are cells in many ways like other cells, and neurobiology has inevitably profited from the application of techniques recently developed elsewhere in cell biology.

Thus there has now burgeoned a flood of work directed at the use of monoclonal antibodies raised by the use of nervous tissue, fractionated in some way or other. The hope is that monoclonal antibodies will be found that are specific for cell-surface proteins which are themselves characteristic of certain types of neurones, perhaps for example making possible distinctions between neighbouring nerve cells with very different functions. Similarly, monoclonal antibodies may make it possible literally to tag the protein molecules of which the receptors specific for neurotransmitters are constructed, thus helping in a close study of their distribution. As elsewhere in cell biology, however, these efforts (some of whose successes are described below) are a little like fishing expeditions.

Still more exciting is the prospect that techniques now widely used elsewhere in cell biology for relating the function of a cell to the chain of information transfer from DNA to RNA to protein may be applicable to neurones, hitherto regarded as improbable candidates for such investigations. For neurones, because they

do not normally replicate, are not expected to be major protein producers. The question whether it may be possible to isolate from neurones species of mRNA that would somehow characterize the functions of the cells from which they are derived, perhaps because they specify receptor protein molecules, was discussed enthusiastically at a meeting at Woods Hole in May. Several investigators returned to their laboratories determined to make the most of suggestions that RNA species in neuronal cells may be relatively few in number, and characteristic of the small number of specific materials produced in neuronal cells.

For the rest, however, one of the surprises of the past few years has been the resurgence of interest and the deepening of interest in two somewhat conventional aspects of the neurosciences — the anatomy of the nervous system and the physiology of the neurone. As always, novel technical developments have been influential, but people have been asking more pertinent questions.

Structures

The anatomy of the mammalian nervous system is of necessity complex. The classical description of the recognizably different structures of the brain has long since made it possible for people to publish atlases showing how different parts are related to each other. Thus the hypothalamus, sitting as it does immediately above the pituitary gland, is physically well placed to be the brain's way of influencing the supply of, say, sex hormones to the body as a whole, and so much has of course been confirmed by the continuing torrent of biochemical studies. But what is the special function of the structure in the centre of the brain known as the claustrum? Such previously cryptic structures are now, fortunately, turning out to have an identifiable role, lending support to the notion that gross anatomical structures that crop up in all normal nervous systems within one species, and which recur in some way or another in other species of the same phylum, are unlikely to be "there" for nothing.

Anatomically, however, the more absorbing questions about the brain are micro-questions. If, for the sake of argument, the human brain has 10^{12} neurones, some of which may make up to 1,000 connections with other cells, it is plainly crucial to know which kinds of cells make what kinds of connections with other kinds of neurones. Biochemical techniques have revolutionized the process of answering such questions, especially in cataloguing the functions of the various regions of the cerebral cortex (where cognitive function seems to be concentrated).

The resurgence of interest in the physiology of the neurone is less a consequence of the development of techniques (which have nevertheless been essential) as of the dawning recognition that if out-

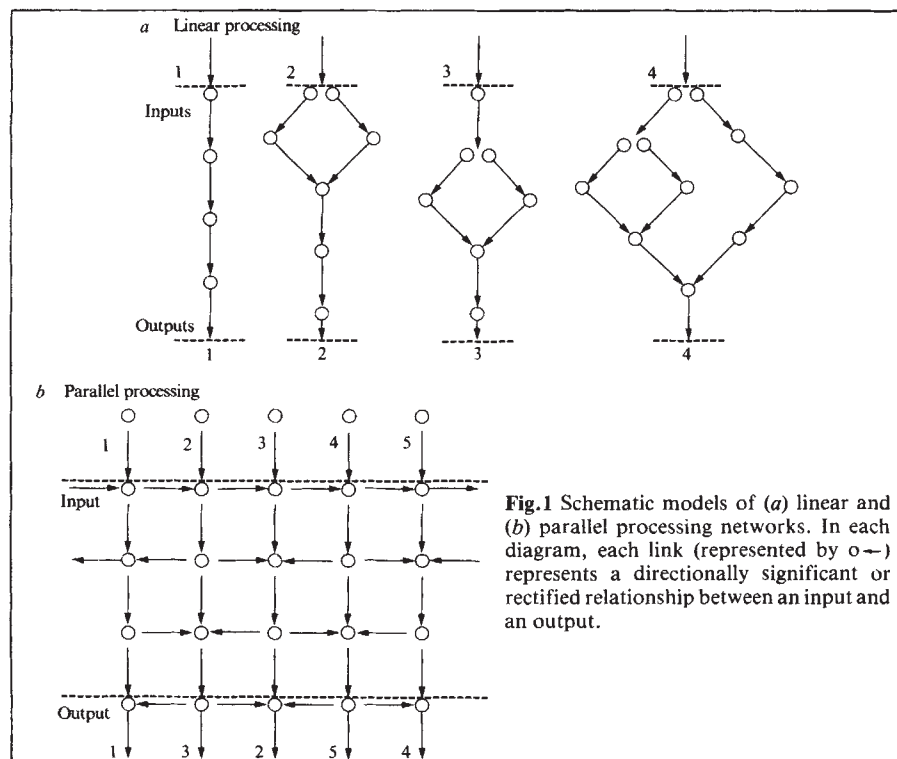


Fig.1 Schematic models of (a) linear and (b) parallel processing networks. In each diagram, each link (represented by $\circ \rightarrow$) represents a directionally significant or rectified relationship between an input and an output.

wardly similar neurones are capable of behaving differently, these differences are likely to be reflected in their physiological properties. This year's fashion, as it happens, is the speculation that Ca^{2+} ions may have an important role in modifying, over a period of days, weeks or months, the physiology of a single neurone: this line of enquiry has an obvious relevance to speculations about the mechanisms of learning and of memory.

In the long run, however, the neurosciences must explain how a collection of about 10^{12} neurones could possibly accomplish the sorts of things that go on in people's heads. The argument that the brain is merely a kind of computer has been advanced ever since the development of computers, and is widely accepted. The neuroanatomists have, however, found that nervous systems differ from conventional (electromechanical) computer hardware in two important respects. First, there is an element of parallel processing in the interlinkage of neurones that does not (as yet) crop up in electronic computers (Fig. 1). Second, although not as malleable as many other kinds of cells, individual neurones are not fixed for all time — a cut-off axon may in favourable circumstances be regenerated, an intact neurone may, without necessarily changing its shape, change its function (the phenomenon of plasticity) as appears to be commonplace during the early development of the nervous system.

So how does the analogy of the computer account for cognition? Most neurobiologists, it must be acknowledged, are suspicious of the analogy, but have little to put in its place. But the computer people are also at a loss to know just how far to take the analogy. Luckily, this field of enquiry has been enlivened by the work of the late Dr David Marr at Massachusetts Institute of Technology, whose algorithm of binocular vision appears to have been confirmed in the psychometry laboratory². So there has been an influential resurgence of the view that the working of a nervous system will be understood only when it has been possible to build an electromechanical model of one.

As things are, these disparate approaches mix like oil and water. Indeed, the hallmark of neurobiology is the way in which the people involved are still imprisoned not so much by their disciplines as by their time-consuming techniques. Much of the work referred to in the following pages has been carried out with experimental systems first conceived of a decade ago, which cannot be ideally pertinent to the questions with which people are now concerned. So it is a good augury for the future that the hard core of neurobiology has been well unified. The Neurosciences Study Program, begun in the late 1950s by Professor F.O. Schmidt of Massachusetts Institute of Technology, has been an important influence in this direction. But there is some way to go. □

Rise and fall of voltage-clamps

VOLTAGE-CLAMPING has become a kind of magic in neurophysiology. Although the principle is simple enough — to keep the voltage across a cell membrane constant — the technology of voltage-clamping has become a sophisticated exercise in electrical engineering. Technically, a voltage-clamp is a negative feedback device — the membrane voltage must be monitored and corrected by means of the output of an amplifier whose signal is the time differential of the voltage. That done, it is merely necessary to measure electrical current.

Such systems are notoriously prone to instability. People worry about the frequency response of the amplifiers they use, the way in which the components of the electronic system are shielded from each other (to reduce stray capacitances in the system) and the exact balance between the resistance of the two micro-electrodes (one inside and one outside the cell) and the impedance of the amplifiers.

The benefits, first recognized by K.S. Cole³ in 1949, are nevertheless substantial. Put simply, the current through a cell membrane is the sum of the currents due to the transport of ionic species and the effective current arising from the non-zero capacitance of the cell membrane, which is proportional to the rate of change of the voltage. One immediate consequence is that it may be impossible to separate the ionic current from that due to the membrane capacitance unless it can be arranged that the transmembrane voltage is constant in time.

Those in the business of voltage-clamping are, however, usually seeking more ambitious objectives. Since the work of Hodgkin and Katz⁴ in 1949, it has been clear that the membrane conductances for K^+ in Na^+ are voltage-dependent, sodium channels opening more quickly than potassium channels. In 1952, Hodgkin and Huxley⁵ used the same technique to study the sequence of events following the depolarization of a nerve cell membrane (which usually encloses a cytoplasm about 65 millivolts electrically negative compared with the exterior).

Their notion that a reduction of the (negative) membrane potential may first increase the inward flow of Na^+ (further

depolarizing the membrane) and then increase the outward flow of K^+ (restoring normality) is now the basis for explaining the passive electrical properties of neurones and the propagation of electrical signals along the processes of nerve cells, axons and dendrites alike. More recently, it has been possible to disentangle the effects of membrane channels supposedly specific for potassium and sodium by means of materials which appear specifically to block the two kinds of channels.

Plainly, the voltage-clamping technique can be used in other fields. Is the effect of specific drugs on neurones principally to affect the conductance of the cell membrane for sodium and potassium? What about the interaction between pairs of neurones — either that mediated by the movement of chemical neurotransmitters or that which appears purely electrical?

In these connections, one of the by-products of the technique of voltage-clamping is that even the noisiness of the measured current has turned out to be of value. For it appears that fluctuations of the current may be related directly to the opening and closing of membrane channels — a point first demonstrated by Katz and Miledi⁶ in 1970 with the motor endplates of muscle fibres activated by the neurotransmitter acetylcholine. Comparable studies with neurones have since provided estimates of the density of sodium and potassium channels in typical membranes and thus of their conductance in molecular terms. The spectrum of current fluctuations is a means of deriving the frequency distribution of the times for which ion channels are open or shut.

No doubt people will be worrying about the best design for voltage-clamping for many years to come, yet for many it will be more rewarding to learn the equally demanding skill (first demonstrated by Neher and Sakmann⁷ in 1976) of working with only a patch of membrane of a neurone; the response of the membrane channels to a transient (microsecond) voltage pulse lasts for much longer than the transient displacement current arising from the electrical capacitance of the membrane. □

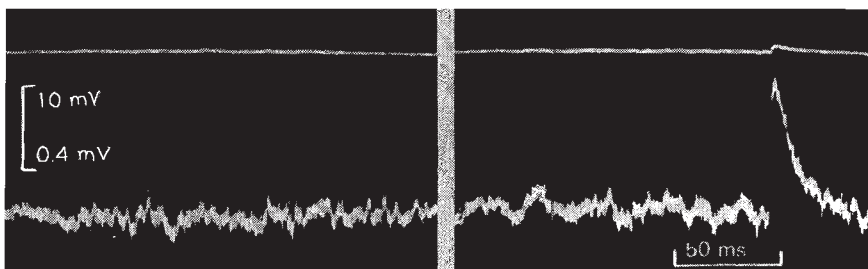


Fig. 2 Low (upper) and high (lower) gain recordings of current across frog muscle endplate under the influence of a steady discharge of acetylcholine from a micropipette. Peaks in the right-hand panel represent spontaneous endplate potential peaks. (Photo: Sir Bernard Katz.)

Revival of neuronal electrophysiology

THAT there should have been a revival of interest in the properties of single neurones is not surprising. As cells go, neurones are unusual structures, able to convert an input signal into an output signal over distances that ordinarily amount to several millimetres and which may be ten times as much. To paraphrase, it might be said that the three most important reasons for the revival are "technique, technique and technique". In reality, there is a little more to be said.

The most influential development has been the penetration through the community of those concerned (and their students) of the coherent view of how neurones function, developed more than fifteen years ago by several people among whom Hodgkin and Huxley are conspicuous. Traditionally, the electrophysiology of neurones has been impenetrable for people working in other fields because the practitioners were often at a loss to know why, for example, a change of the external voltage of a neurone would trigger electrical activity within the

neatly explained by the superposition of two opposing flows of Na^+ and K^+ , the first of which grows more quickly than the second. So attention has naturally turned to investigations of the properties of the channels in neuronal cell membranes that effect the transfer of sodium and potassium ions (and of others as well). That their conductance should be voltage dependent, well attested by experiment, remains to be accounted for in molecular terms.

Similarly, the propagation of electrical signals along the length of the long processes of nerve cells, axons for example, has been cast into terms easily recognizable by electrical engineers concerned with transmission lines. The electrical properties of the cell membrane are the equivalents of capacitance and resistance connected in parallel and distributed between two more or less parallel conducting transmission lines — the cytoplasm and the cell exterior (see Fig. 3). So the electrical properties of a neurone may be connected, at least in principle, with the molecular properties of its constituents. Neat explanations of familiar observations tumble out — neuronal axons wrapped in myelin (a dielectric) have lowered capacitance and thus (according to the electrical engineers) propagate signals at a heightened speed.

None of this, of course, is intended to suggest that there is a well tried model of neuronal function that has been confirmed in detail; rather, there is a model of how neurones function that happens to be consistent with certain familiar phenomena and which is powerfully suggestive of investigations that may throw light on the more detailed behaviour of different types of neurone. The development has something in common with the stimulus provided for chemists by the invention of the Periodic Table.

New ways

The study of these phenomena has been transformed in the past few years by the development by Neher and Sakmann⁷ of a technique for measuring the movement of ions through small patches of the membrane of a cell. The technique involves the use of a micropipette into which (in the most recent version of the technique) a patch of membrane is sucked so as to make a seal whose electrical resistance is very large (see ref. 8 and Fig. 4). As a result, leakage is minimized.

Neher and his associates have been able to show (first with muscle fibres): that patches about $1\ \mu\text{m}$ in diameter may contain small numbers and perhaps only a

single channel that can be opened by the influence of the neuromuscular transmitter acetylcholine; that such a channel will remain open for several milliseconds before closing spontaneously; that the times for which the same channel is open are statistically distributed in an exponential fashion; and also that a channel will spontaneously become desensitized to acetylcholine after some seconds of activity, but that it will afterwards spontaneously recover its activity, again on a time scale of seconds.

The development of techniques in electrophysiology, quite apart from giving currency to the verb "to voltage-clamp", has also made it possible to extend studies of neuronal function from exceptional neurones — the giant axon of the squid, for example — to neurones whose behaviour is more likely to resemble that of the mammalian nervous system. The same measurements can yield estimates of the strength of ionic currents. Delicate neurophysiology of this kind is not yet possible with neurones that are parts of intact nervous systems, however.

These techniques have made it possible to confirm earlier estimates of the capacity of ion channels — when open, they appear capable of transmitting some 15,000 to 30,000 ions each millisecond. The time taken for an acetylcholine molecule to provoke the opening of a channel (too small to be resolved directly) is probably a few microseconds. There is evidence⁹ that acetylcholine receptors isolated from the electric organ of the electric fish *Torpedo mormorata* function in the same way, even when incorporated into synthetic lipid bilayers.

Where next?

In these early days, the importance of this technique cannot easily be guessed at. Muscle fibres are not necessarily representative of neurones as such, but there is no reason why these observations should not be extended (by those who have mastered the technique). There have been some suggestions that, while the observed properties of single ion channels are qualitatively consistent with the Hodgkin-Huxley picture, some apparent quan-

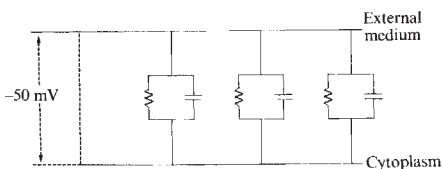


Fig. 3 Transmission line equivalent of conduction along a nerve process. The parallel resistors and capacitors represent the membrane resistance and capacity for an infinitesimally short length of cell process (and there is an infinite number of them). One complication in computation is that the resistances are voltage dependent.

cell (and sometimes suppress it).

Neurones, like other cells, are devices whose special properties derive from mechanisms by which they contradict the requirements of thermodynamics. (Entropy is by no means a maximum.) Neurones are primarily cells in which the internal concentration of K^+ is greater than would be expected from the external concentration of this ion outside the cell, while the Na^+ concentration is less, and, even when passive, neurones must expend metabolic energy to maintain this state of affairs.

That changing the voltage across the cell membrane (which encloses a space which is electrically negative by about 50–60 mV) might have the dramatic effects observed classically in electrophysiology — a brief spike of current inwards across the cell membrane followed by a less intense current in the opposite direction — is

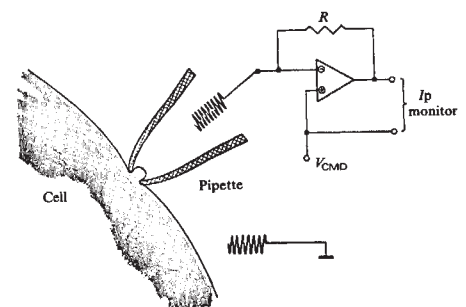


Fig. 4 Schematic diagram of patch clamp measurement. The diameter of the pipette may be from 0.3 to $0.5\ \mu\text{m}$. (From Sigworth and Neher, *Nature* **287**, 447; 1980.)

titative discrepancies may be significant.

Those who hope for the best look forward to the use of the patch technique for the direct measurement of the properties of neurones too small to be measured by means of intracellular electrodes and for distinguishing between

the effects of different extracellular agents (including drugs) on the permeability of the cell membrane. At least one experiment¹⁰ suggests that the effects of local anaesthetics on the cell membrane may be to interrupt the flow of ions through a membrane channel, perhaps by physically

blocking it, perhaps by some special interaction with the receptor. The application of the same technique to detached patches of cell membrane offers a way of exploring the intracellular influences on ion transport through neuronal membranes. □

Making potassium run uphill

THE mechanism that concentrates potassium ions within neurones (and other cells), the sodium–potassium pump, has recently been given a degree of notoriety because of the suggestion by Professor Efraim Racker's group at Cornell University¹¹ that phosphorylation of the enzyme responsible might be the crucial step in a cascade of biochemical reactions leading to malignancy. Although frequently overshadowed by the hunt for active receptors on neuronal cell surfaces, the characterization of the molecular components of the pump has made good progress in the past decade, but controversies and uncertainties persist.

Like the contents of other kinds of cells, the contents of neurones are in profound chemical disequilibrium with the extracellular space. It is therefore a little odd that while there is great interest centred on the search for neurotransmitters that will help to undermine this state of disequilibrium by making neuronal membranes more permeable to ions, the mechanism that maintains the disequilibrium appears to be regarded as a somewhat traditional branch of biochemistry — but is not well understood on that account.

Part of the trouble may be that the agent responsible has been recognized as a protein and as an enzyme bound in the cell membrane, but was originally known by the most un-descriptive (and misleading) name in the biochemists' vocabulary — that of a K^+ - and Na^+ -dependent membrane-bound ATPase. Although more is now known of this pump, the true nature of this essential constituent of the cell membrane is far from clear.

Uphill work

Another reason why the sodium–potassium pumping mechanism receives less attention than it deserves is that it plays a crucial part not merely in glamorous organs such as the nervous system but also in muscles, conspicuously (in everyday clinical experience) in those of the heart, whose supply of potassium must be artificially made good following the use of glycosides such as digitalis in heart disease.

In all cells, the normal function of the pump is to extrude Na^+ and to take in K^+ ions. Even in resting cells, leakage of the ions in the direction of thermodynamic equilibrium is continuous, although not as

great as when they are depolarized. Ion transport by the sodium–potassium pump must be sufficient, in the resting cell, to balance passive leakage, but must also be capable of restoring the loss of potassium, and of removing the unwanted increment of sodium, after a period of activity by a nerve (or muscle) cell.

The normal resting voltage of a neurone is a direct measure of the free energy involved in the process — the height of the hill up which water (represented by potassium) must be made to flow. Physical chemists will recognize that the voltage corresponding to the maintenance of K^+ concentrations inside and outside the membrane of, say, C_1 and C_2 is given by the Nernst equation as

$$RT/F \ln(C_2/C_1)$$

where R is the gas constant, F the Faraday constant and T the temperature. To account for the maintenance of multiple ion-gradients, the concentrations in this equation must be replaced by those of all the ion species weighted according to their permeabilities through the membrane. In the resting neurone, this entails a slight reduction of the voltage due to potassium disequilibrium that would otherwise obtain.

The pump

The essential ingredients of the Na^+/K^+ pump are two protein molecules, one a hydrophobic protein of about 120,000 daltons and the other a glycoprotein of 55,000 daltons (see Fig.5). The two components are closely associated, and it appears to be common ground that equal numbers of the two molecules are involved in transporting Na^+ and K^+ , that the

protein is embedded in the cell membrane and that its effectiveness in transporting sodium (normally outwards) and potassium (normally inwards) involves both phosphorylation (by ATP) and a conformational change of the enzyme.

There is, however, disagreement about whether the pump exists in membranes as a dimer of a linked pair of protein molecules (see refs 12, 13) and some doubt whether the customary view that the export of sodium is followed sequentially by the import of potassium must be replaced by the view that the two cations are transported more or less simultaneously. Participants at a conference at Yale University earlier in the summer appear to have been persuaded that simultaneous transport may now be more plausible.

Either way, the phosphorylation of the enzyme is accomplished by the transfer of one phosphate group from a molecule of ATP — a process for which Na^+ is necessary — and the subsequent dephosphorylation (catalysed by K^+). Transmembrane transport appears to involve the sequestering of ions within the structure of the protein and the kinetics and thermodynamics of the process appear to be consistent with the export of three Na^+ and the import of two K^+ ions for each cycle of phosphorylation and dephosphorylation.

With the help of sodium–potassium pumps in special systems, red cell ghosts for example, it has been possible to demonstrate¹⁴ that the pump can be made to operate abnormally in suitable circumstances, pumping either Na^+ or K^+ alone, or even operating backward: (which merely confirms the inference from the Second Law of Thermodynamics that Maxwell's demon does not exist).

Several important issues remain unanswered. What, for example, is the significance of the close molecular homology around the site of phosphorylation (an aspartic acid residue) in the sodium–potassium pump and the analogous pump for Ca^{2+} ? It would be no great surprise if these analogous enzymes had a common evolutionary origin. What are the mechanisms whereby the mechanism of the pump may be blocked by drugs, glycosides for example? This question is clinically significant, as is the open question whether the pump has some relevance to the use of lithium in the treatment of manic depressives. □

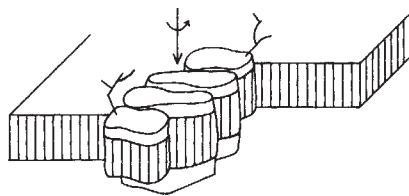


Fig.5 Drawing of the imagined molecular structure Na^+ - and K^+ -dependent ATPase. The subunits are drawn so that their volumes correspond to molecular weights of 120,000 and 55,000 using as a scale the width of the bilayer (4.0 nm).

Passing messages downstream

ONE of the successes of the past few years in neurophysiology has been the description of how neurones communicate with their neighbours, as they must in any properly functioning nervous system. Whether the medium of communication is strictly electrical (literally the movement of ions across a gap junction) or the release of a chemical transmitter at a synapse, the immediate consequence is to increase the permeability of the postsynaptic membrane to the ions principally involved, Na^+ and K^+ . In the membranes of postsynaptic cells at gap junctions, the normal channels of permeability are sufficient. In chemical transmission, other distinct characteristics are involved.

So much has been demonstrated unambiguously by the technique of patch recording developed by Neher and his colleagues⁷ but has earlier been demonstrated by Katz and Miledi¹⁸, at least for those forms of signalling between neurones in which the presynaptic cell excites a positive response downstream.

The neuromuscular junction

At the neuromuscular junction (where the neurotransmitter is acetylcholine), exposure of the muscle cell to transmitter can be recognized by the opening of sodium and potassium channels for periods measured in tens of milliseconds. More recently, Neher and Steinbach have

shown that the period for which the channels are open can be increased by the use of analogues of the transmitter.

That the consequences of such an increase of permeability should tend to excite the postsynaptic cell is easily understood. For if such a cell is even partially depolarized electrically, the flux of Na^+ and K^+ ions is increased.

Thus, to some degree, the effects of a neurotransmitter on the membranes of a postsynaptic cell mimic the state of affairs obtaining during strictly electrical depolarization. Provided that the increased permeability is sufficient to correspond to the threshold voltage at which a cell will spontaneously produce (and propagate along its length) an action potential (a spike of voltage, or a pulse of current), even a chemical signal from a presynaptic cell will cause the postsynaptic cell to fire.

There is, however, endless scope for subtlety in this interaction. If the effect of a

Electrical connections

WITH the excitement of the past several years at the discovery of new chemicals or classes of chemicals capable of effecting communication between neurones, people have tended to overlook the importance of strictly electrical communication between neurones. They should not. The 1920s controversy between Sir Henry Dale, who argued that synapses (the points at which neurones approach each other closely) are bridged by neurotransmitters, and Sir John Eccles, who argued that electrical current did the job, has been settled as a drawn game. (Ironically, Eccles gave in at about the time that Fatt and Katz¹⁵ provided conclusive evidence in his support.)

Both methods of communication are common. One of the surprises of the past year or so is that electrical communication has been demonstrated in the mammalian hippocampus, hypothalamus and olfactory cortex, mostly by electron microscopy but in some investigations by physiological means.

Two features of such an electrical junction are distinctive¹⁶. First, the distance between the outer surface of the two neurones is an order of magnitude smaller than that across the typical chemical synapse, and may be merely 2 nm (20 Å). Second, electron micrographs suggest a visible gap in each of the membranes placed so that the cytoplasm of the two cells may be thought to be in direct communication.

Electrical synapses are not, however, simply holes in neighbouring neurones that happen more or less to coincide with each other. Largely on the basis of electron micrography, it appears that such gaps are spanned by well-ordered

hexagonal structures made of molecules of a protein called (no doubt for want of a better name) connexin.

The basic constituent of connexin is a protein molecule of about 27,000 daltons, whose amino acid constitution is well on the way to being determined. Hexamers of connexin form dumbbell-shaped structures which double up, end to end, form "connexons" that span the gap between neurones, and are themselves arranged in a roughly hexagonal pattern (see, for example, Unwin and Zampighi¹⁷).

Electrical synapses have the advantage of speed. A signal in the presynaptic cell is a pattern of positive charge travelling along the cell process at a speed determined primarily by the capacitance of the cell membrane and the electrical conductance along the process.

At electrical synapses, where it is supposed that the inter-cell electrical conductance is high compared with that across the membrane, there is no reason why the wave of depolarization should not be projected directly into the postsynaptic cell. Moreover, the process is fast and a signal can be transferred from one cell to another without being spread out in time or made less sharp.

In many of their roles, electrical junctions may be important because they allow two-way communication. Such reciprocal signalling may be necessary when groups of neurones are required to function in concert. The increased frequency of electrical communication in cold-blooded animals such as fish suggests that it may be an adaptation to low-temperature operation, when the rate of cell metabolism on which chemical signalling depends is reduced. But

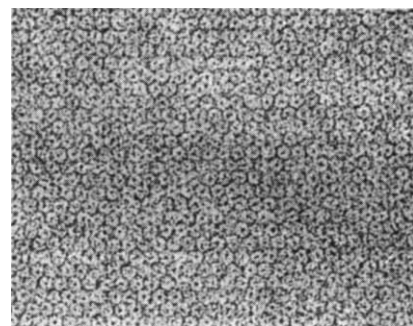


Fig. 6 Electron micrograph of isolated gap junction sheets viewed from a direction perpendicular to the plane of the membranes. The connexons are seen as annular units organized on a hexagonal lattice, with unit cell dimensions 85 Å. Uranyl acetate negative stain.

devotees insist that electrical signalling is not phylogenetically primitive — it does not occur in protozoa for example.

Moreover, it also seems that the efficiency of signalling across a gap junction is not simply a function of the shape of the gap, but that it can be regulated by physiological changes in the cell medium, especially by variations of pH and, less well, by the concentration of Ca^{2+} . Those working in the field hold that the prevalence of electrical signalling between neurones is at least as important as the discovery of a new class of chemical neurotransmitters.

Indeed, electrical communication is surprisingly common. In the past year or so, gap junctions have been demonstrated in the mammalian hippocampus, hypothalamus and olfactory cortex, usually by means of electron microscopy but in some cases physiologically. Previously, electrical junctions had been recognized mostly in the peripheral nervous system — the spinal column, retina and so on.

neurotransmitter at a synapse on the post-synaptic cell is, for example, to increase the permeability of the membrane to K^+ ions, the consequence will not be excitation but inhibition — the potential across the post-synaptic membrane will be increased (made more negative) and the chance that the cell will reach the threshold at which signals are propagated will be decreased.

In any case, the membrane channels for sodium and potassium opened by neurotransmitters are distinct from those normally responsible for membrane permeability and are, in particular, not voltage sensitive. In many cells, the influx of sodium and the efflux of potassium are simultaneous and roughly balanced stoichiometrically, so that the effect of a neurotransmitter is rather like that of a sodium-potassium pump forced to run backwards. (But the sodium-potassium pump is too slow.) The consequences for the chance that the postsynaptic cell will produce an action potential, a spike of voltage, are determined by the physical distribution of the different types of channels in the process of the postsynaptic cell carrying the synapse.

Neuronal conduction

Computer engineers will recognize that these properties of chemical synapses are a means by which physiological analogues of familiar computer circuits may be constructed. Neurones normally carry several synapses on the same process (say a signal-collecting dendrite) and have several such processes running to the main body of the cell. Whether the output end of the cell will then transmit an onward signal depends not merely on the strength but on the phasing in time of the several input signals.

The existence within a single neurone of the equivalent of either "AND" or "OR" circuits is thus easily visualized. The pattern of dendrites leading to the cell body determines what functions the cell is capable of. Different functions, "AND" or "OR", are possible if some of the input signals are null.

Given the complexity of the standard neurone — and those in the motor system of vertebrates are known to have as many as 1,000 input channels — there are many more than two logical alternative choices possible. But it appears now to be established that whether a neurone will transmit an onward signal or not is determined at a point at the base of the axon where the depolarization needed to trigger a signal is comparatively low, more like 15 mV than the usual 30 mV.

This brief outline of neuronal conduction is inevitably an over-simplification. For example, it is now known that the excitation of a postsynaptic cell involves the inward transport of Ca^{2+} (ref.19); although the amounts of these ions transported are two orders of magnitude smaller than those of Na^+ and K^+ (in the squid and frog neuromuscular

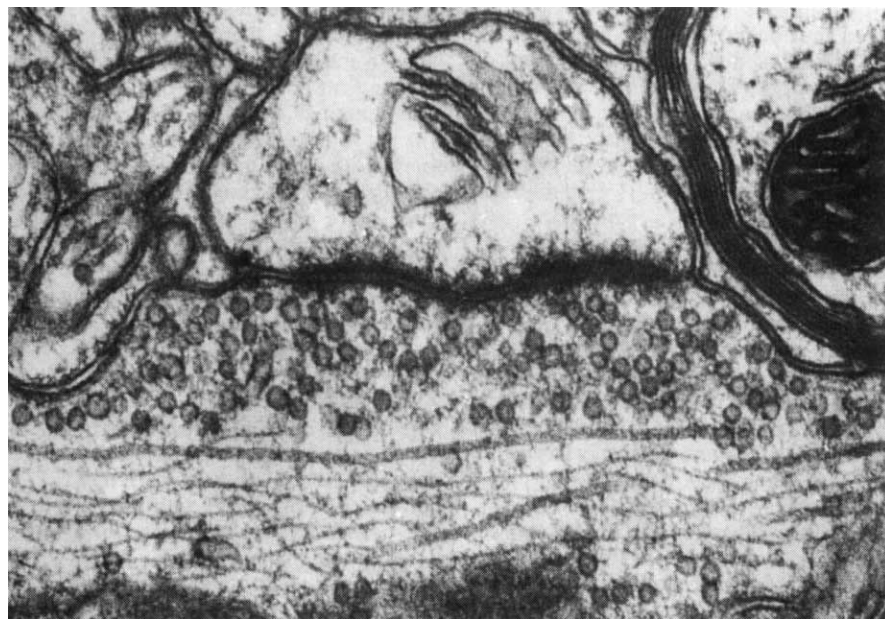


Fig. 7 Electron micrograph of a synapse in the rat cerebral cortex between a dendrite and an axon. The axon can be seen passing along the bottom half of the figure, forming *en passant* a varicosity, or bulge, which contains numerous vesicles believed to hold neurotransmitter molecules. The dendrite lies above the varicosity, its membrane closely apposed to that of the axon. (Photo: Dr P. Gordon-Weeks.)

junctions examined) it is not likely that the Ca^{2+} will be without physiological significance.

Several questions (apart from the possible influence of other ions) remain, however, unanswered. The most urgent need is an understanding of the molecular structure of the two kinds of ion channels, those activated by neurotransmitters and those which are normal constituents of neuronal membranes. The acetylcholine receptor offers the most immediate hope under the first heading (see below). The prospects for understanding the mechanism of the voltage-gated channels that account for the flux of Na^+ and K^+ during normal activity of a neurone must await some means of isolating substantial

amounts of them. Will cloning help?

The distribution of transmitter-sensitive receptors away from the region of the synapse is also unclear — and a potential problem. Denervated neuromuscular junctions show that sodium-potassium channels activated by neurotransmitters occur all over the surface of the postsynaptic cell. It would be a great surprise if the protein receptors responsible (when activated by a molecule of neurotransmitter) were all gathered into synaptic junctions. So must it be supposed that neurones are susceptible not merely to the highly directed processes of communication at synapses but to generalized influences as well? Most probably yes. But how? Nobody knows. □

What transmitters do

THERE are known to be several ways of skinning a cat and, similarly, there are several ways of inhibiting a neurone from firing. So much, for example, is now well demonstrated for the neurotransmitter called γ -aminobutyric acid (GABA for short), a simple amino acid closely related to glutamic acid — it lacks a carboxyl group. Several lines of enquiry suggest that the immediate effect of GABA on neurones is to increase the permeability of the cell membrane to Cl^- ions, normally at a lower concentration in the interior of a neurone than in the surrounding medium.

The most direct demonstration of this function has been provided by studies

carried out with model systems, for example with single neurones obtained by growing the embryonic rat spinal cord in culture, a system developed at the National Institutes of Health by Dr Geoffrey Barker. (Similar systems based on the use of other embryonic tissue have been used elsewhere.) The universal objective is a collection of model neurones from which voltage-clamped single-cell recordings or patch recordings can be made.

Possible mechanisms

Any mechanism that allows the inward flow of Cl^- ions must increase the (negative) electrical potential of the

cytoplasm of a neurone, *hyper*-polarizing it and thus decreasing the chance that the cell will be excited. But the responses of individual cells in cultures of rat embryo spinal cord neurones vary between cells, suggesting that the inhibitory effects of GABA depend either on the numbers of Cl^- channels that happen to be embedded in the intact membranes of the model cells or perhaps even on the degree to which the channels are clumped together, as supposedly in a synapse.

More recently, the inhibition of postsynaptic response by GABA has been confirmed by the newer technique in which slices of nervous tissue are used for making physiological recordings in conditions that more closely simulate what may happen in real life. Thus Alger and Nicoll²¹ have confirmed the inhibitory effects of GABA, but have also suggested that the mechanism is unlikely to be exclusively to open Cl^- channels in the membrane; perhaps Ca^{2+} channels are also involved.

Parenthetically, it is surprising that the use of slices of nervous tissue for the study of the properties of neurones should have been introduced only in the past five years, by P.O. Andersen at Oslo^{22,23}. After all, a good deal of biochemistry has been made possible, in the past several decades, by the use of slices of tissue from the liver and other organs. Why not the nervous system? One practitioner confesses that the explanation may have been the psychological aversion of people like himself to slicing up the brain or anything connected with it.

Other neurotransmitters

The inhibition of the postsynaptic cells by means of channels that increase the conductance of Cl^- ions has also been confirmed for putative neurotransmitters other than GABA, for example, the amino acids glycine and β -alanine. Indeed, there are suggestions that the Cl^- channels are identical for all three amino acids²⁴.

Other neurotransmitters which inhibit postsynaptic neurones appear to function in different ways. Thus there is evidence that, in the hippocampus, serotonin (another of the eight classical neurotransmitters) inhibits postsynaptic cells by increasing the permeability of the membrane to K^+ . The peptide neurotransmitter enkephalin appears to have a similar effect in the tissues of the locus coeruleus in the mammalian brain. Similarly, glutamate (ionically neutral glutamic acid) excites postsynaptic cells by increasing K^+ conductance, but the peptide neurotransmitters inhibit that effect.

Drug action

Such studies have also provided the beginnings of a rudimentary understanding of how some psychotropic drugs may function. Thus there is evidence that

pentobarbital, an anaesthetic, may have a similar effect to that of GABA but one which is more prolonged, producing long-term inward conductance of Cl^- and thus inactivation of the neurones concerned²⁰. The benzodiazepines have also been shown to affect the conductance of Cl^- channels, although pharmacological studies showing that there are also receptors in the mammalian nervous system specific for these materials suggest that increased Cl^- conductance may not be the only effect of their use.

Problems

The burden of such studies is that there is no simple way of enumerating the responses of a postsynaptic cell to any but the simplest of neurotransmitters. First, the transmembrane current that determines the response consists not merely of flows of positive ions such as Na^+ , K^+ and, to a lesser extent, Ca^{2+} , but

of Cl^- ions as well. And while some of the specific receptors implied by these phenomena are plainly also specific for certain neurotransmitters, others merely have their action modified by secondary transmitters — the influence of the peptide neurotransmitters on the glutamate-sensitive K^+ channels for example.

In such complicated circumstances, there is bound to be a certain uneasiness about the applicability of results derived from the study of model systems to what happens in the real nervous system. A few simple truths stand out, however; plainly there is a need to know more about the channels responsible for Cl^- conductance as well as for the interaction of two different kinds of neurotransmitter at the same receptor. A study²⁵ of the effects of opiate peptides and analogues thereof on the inhibitory responses of postsynaptic cells also suggests that the distribution of their receptors, rather than the total numbers of them, may be crucial. □

Calcium helps to send out signals

By what means can a voltage pulse in a presynaptic neurone stimulate the release of a package of neurotransmitter molecules at the synapse? Ordinarily, electrical phenomena at the level of millivolts and chemical phenomena do not mix. What, at synaptic junctions, can be responsible? For some years, this has been the central problem for those concerned with the mechanism of synaptic action. Half the answer now seems clear. What determines the release of neurotransmitter from a presynaptic cell is the influx of Ca^{2+} within the neighbourhood of the synapse.

Synaptic structure

The general structure of synapses has been known for several years, and is easily rationalized. Electron micrographs show that presynaptic junctions are well endowed with mitochondria, a sign of active biosynthesis. They are also well populated by vesicles shown (by studies, for example, with radioactive tracers) to be repositories of neurotransmitters. Physiological experiments (involving the effects on postsynaptic cells) show that packages of neurotransmitter are released as discrete quantities, giving a certain noisiness (described by physiologists as a "quantum" effect) in the response of the downstream cell.

Most probably, the same general model will serve for the secretion of any humoral substance by any secreting cell. The

distinctive feature of the synaptic secretion of neurotransmitter is that the process is concentrated across a narrow region of the cell surface, and that secretion is stimulated electrically. How? The simple answer is that Ca^{2+} ions are responsible. The argument is as follows.

The role for calcium

The model system is again that of the neuromuscular junction, mediated by acetylcholine. The founding study is that of Katz and Miledi²⁶, who showed that the release of neurotransmitter at a neuromuscular junction typically involves the discharge of more than 5,000 molecules of acetylcholine into the intervening space. Earlier, by a process of elimination, they had shown (using systems in which either K^+ or Na^+ channels, or both, had been blocked) that neither of these ion species is essential for making sure that a voltage pulse will lead to the release of a package of neurotransmitter. Ca^{2+} was the obvious alternative.

This conclusion has now been amply confirmed, principally by Kandel's group at Columbia University, New York. The issue is relatively simple. The arrival at a presynaptic junction of a positive (less negative) voltage pulse will certainly stimulate (as always) an influx of Na^+ , and a few milliseconds later an efflux of K^+ . But at the ends of neuronal processes, and

on this very simple picture, nothing else can happen. Surprisingly, as early as 1967, Katz and del Castillo²⁷ had demonstrated not merely that Ca^{2+} influx is habitual at synaptic junctions but that Ca^{2+} influx is essential for the release of acetylcholine at neuromuscular junctions — still the only easily accessible model of a specific synapse.

A possible function

Kandel and his associates have been able to endow the movement of Ca^{2+} across the presynaptic membrane with more subtle significance: briefly, there seems to be a connection between the fluxes of Ca^{2+} ions under various circumstances and long-term modifications of cell behaviour, linked possibly with the phenomenon of learning and of memory.

As things are, however, nothing is known of the function of Ca^{2+} ions in presynaptic neurones except, phenomenologically, that they are necessary and perhaps even sufficient for transmitter release. Plainly there is a task ahead for the biochemists to work out what happens. Fortunately they are now equipped with the necessary tools: calcium chelating agents and also ligands that, when bound to calcium, will fluoresce. □

Benefits of tissue slices

THE technique which depends on the use of tissue slices, introduced in the past five years by Professor P.O. Andersen of the University of Oslo, appears to have its origins in the work of Professor H.C. McIlwain in the early 1960s. Although principally interested in the biochemistry of nervous tissue, McIlwain made electrophysiological recordings from nerve cells in the samples he prepared, only to find that physiologists complained that the recordings could not be physiologically significant. McIlwain's use of brain slices was, however, developed by C.D. Richards at the National Institute of Medical Research of the Medical Research Council.

Andersen's development of the technique depended on the recognition that the cells of the hippocampus form an exquisite sequence of lamellae, so that the process of cutting slices through the hippocampus need not sever too many neuronal processes. Now the technique is being applied to other parts of the central nervous system where regular structures are expected, the olfactory lobe for example, but also the columnar structures in the visual cortex.

Properly incubated slices of tissue will remain physiologically active for several hours. For practical purposes, the technique offers the only effective way of studying the effect of the excitation of one neurone on others lying downstream.

Towards understanding the acetylcholine receptor

THE acetylcholine receptor, the workhorse of classical physiology, is likely to be the first of the neuronal receptors to be subjected to the full treatment of molecular biology. The explanation is straightforward. Species of the electric eel *Torpedo* use arrays of the receptors as means of generating the electric fields with which they can surround themselves, and are thus potentially rich sources of messenger RNA specifying the protein content of the receptor.

Several groups are occupied with two applications of modern techniques to this RNA. First, systems are being developed for the translation of the messenger RNA in cell-free systems, so as more fully to characterize the protein constituents of the receptor. Second, by using the mRNA as a template for generating the corresponding stretches of DNA and cloning the product, several groups hope to accumulate enough material to work out the nucleotide sequence corresponding to the mRNA. It is still not clear whether those concerned will

be ready to announce their results at the meeting of the Society of Neurosciences in Los Angeles next week.

Meanwhile, a great deal of interest has been stimulated by the report²⁸ of a high degree of amino acid sequence is constructed. Approximately, the four distinct units have molecular weights of 40,000, 60,000 and 65,000, and the intact receptor includes two of the lightest units.

The sequence of amino acid residues at the amino terminal end of each of these units, spanning some 56 residues altogether, contains enough common elements arranged in the same order as to suggest that they evolved from a common gene. Was the primitive receptor an aggregate of five identical subunits, and does that suggest a way of tackling the problem of the function of the modern receptor? No doubt it is only a matter of time before putative primitive structures are synthesized.

The importance of this development is not easily underplayed. There is every

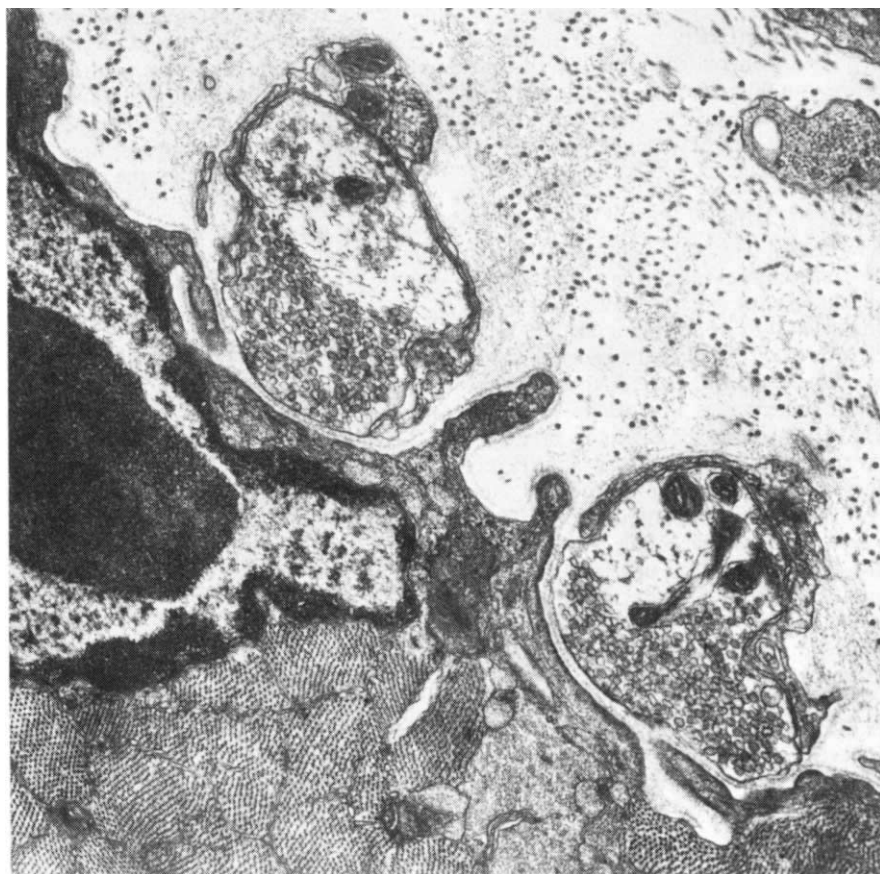


Fig. 8 Electron micrograph showing two frog neuromuscular junctions. Two nerve terminals can be seen, lying close to the muscle membrane. They contain vesicles of the neurotransmitter acetylcholine. Muscle fibres can be seen in the bottom left-hand part of the figure, running out of the plane of the paper. (Photo: Dr P. Gordon-Weeks.)

reason to suppose that the structure of the receptor in the electric eel *Torpedo* is similar to that of the same receptor in mammalian tissue. And if it should be possible to tell, with the help of X-ray analysis of crystallized receptors, how the channels that admit Na^+ and K^+ to pass through the membrane when the receptors are attached to a molecule of acetylcholine, there is every chance that a model will have been provided for other ion channels in membranes, those which are activated by a change of membrane voltage as well as those which respond to neurotransmitters.

This goal will not, however, be easily attained. In the past five years, the structure of the receptor and the charac-

teristics of its operation have been reasonably well defined, yet a model of the mechanism remains elusive. The protein, which spans the membrane (protruding about 5 nm from the outer surface and 1.5 nm from the inner surface) has a diameter of 8.5 nm and consists of five polypeptide subunits, the two smallest of which (40,000 daltons) are responsible for binding acetylcholine.

Electron microscopy suggests that the external part of the receptor is funnel-shaped, and the ion channel is thought to be 0.65 nm in diameter. The dynamics of receptor function have now been characterized both for naturally occurring receptors and with model systems in which the

receptors are embedded in artificial lipid membranes^{29,30}: activated channels remain open for an average of about 1 millisecond. The acts of opening and closing are accomplished in periods of the order of a microsecond.

The precise difference between an activated and an inactive channel remains, however, to be described. Fortunately, there is now a wide range of pharmacological agents that will interfere with the normal operation of the receptor, so that a systematic exploration of the behaviour of the receptor in response to various agents may suggest ways in which the conformation of the ion channel can be manipulated. □

New classes of neurotransmitters

NOWHERE in the neurosciences do fashions come and go more quickly than in the hunt for materials that may function as neurotransmitters. Thus for example there has been great excitement in the past few years about the discovery in nervous tissue of simple peptides which have an affinity for particular receptors and which have therefore been assumed either to be neurotransmitters themselves or the precursors of them. The difficulty is that as the list of peptides has increased in length — there are now more than 25 of them and another is reported on page 579 — their function has become less well determined, even embarrassingly vague.

Enkephalins

The first of the brain peptides to have a pharmacological effect are two simple pentapeptides now known as enkephalins³². For these materials, at least, there does appear to be an explicit pharmacological function. Indeed, the discovery of the enkephalins was prompted by the study of the receptors in the brains of experimental animals which specifically bind morphine and similar opiates, and whose physiological consequences for nervous tissue are reasonably well defined. The discovery that the drug naloxone will specifically prevent binding of morphine to the opiate receptors was of great assistance in the identification of the naturally occurring enkephalins, now known as Met- and Leu-enkephalin after the amino acids at their amino termini.

Developments since 1975 have been bewildering. First, it appears that the amino acid sequence of the enkephalins is contained within that of many other peptides since isolated from nervous tissue, for example in the peptide β -endorphin, itself contained within the amino acid sequence of β -lipotropin. Second, while there is some evidence that other brain peptides, in par-

ticular neurotensin and substance P, have specific interactions with nerve cells in compact areas of the brain of laboratory animals, and that substance P in particular is involved with the sensation of pain, there are as yet few studies that demonstrate conclusively that these materials function as neurotransmitters in the classical sense.

The issue is inevitably controversial. The difficulty in resolving it stems from the way in which most studies of the presence of putative peptide neurotransmitters in nervous tissue are based on techniques such as using immunofluorescence antibodies against the peptides to suggest how they may be distributed in, say, the rat brain. Inevitably, a demonstration that a particular peptide may be localized in some limited region of the brain does not constitute a proof that it functions there as a

neurotransmitter; for that purpose much more specific investigations will be necessary.

If it should turn out that many of the materials so far described are not neurotransmitters in the classical sense of binding to specific receptors and then producing a well defined physiological consequence, it is nevertheless more than probable that the brain peptides play some other role in the organization of nervous function. This is why some of those working in the field consider that there may be a similarity between the secretion of neuropeptides from generalized areas of the brain and the secretion of the hormones that regulate the pituitary gland from the hypothalamus.

Purines

If, however, the past few years have seen a decline of enthusiasm for the view that the neuropeptides are specific neurotransmitters, another fashion is already on the rise. In the past decade, Burnstock³³ in particular has argued that purines such as

Explaining myasthenia gravis

THE most startling property of the acetylcholine receptor to have come to light in recent years is its involvement in the autoimmune disease myasthenia gravis, a defect of neuromuscular transmission that particularly affects the muscles of the face, in which there is no obvious evidence of the degeneration of the nerve supply to the muscles affected and whose symptoms can be partially ameliorated by the use of drugs analogous to acetylcholine. Since the 1930s, when the removal of the thymus was first introduced as the standard therapy for the disease, it has been accepted that the disease has an immunological origin.

The clinching observation (in 1973) was that of Patrick and Lindstrom³¹ that the injection of acetylcholine receptors from a species of electric eel into rabbits (intended as a means of raising antibodies

to the material) caused the animals to fall sick with symptoms analogous to those in the human disease. Since then, it has been amply confirmed that myasthenia gravis is an autoimmune disease in which circulating antibodies against a person's acetylcholine receptor molecules have somehow arisen.

With all this said, there are several outstanding problems. The causation of the production of circulating antibodies is not understood, nor the mechanism of the interaction of antibody with the intact receptor molecules. Nor is it understood why some patients with severe symptoms of the disease lack circulating antibodies, and why the effects of the antibodies vary from one muscle to another. The result, so far, is that there have been no radical improvements of therapy to match the improvement of the understanding of the disease. □

adenosine, the nucleotide in ATP, are potent and specific neurotransmitters in a variety of tissues. The case has been hard to prove, but it does, however, appear that in the autonomic nervous system, there are at least some nervous pathways which are activated by neither acetylcholine nor noradrenaline. These are the type specimens of the neuronal circuits supposed to be activated by the purines.

Moreover, it appears to have been possible to identify at least two different classes of purine receptors — those which are more responsive to adenosine and adenosine monophosphate (AMP) and which are known as P_1 receptors; and those which are more responsive to ATP and adenosine diphosphate (ADP), which are known as P_2 receptors.

Possible functions

The work so far carried out suggests that one of the functions of the adenosine receptors may be to control the activity of, say, acetylcholine-releasing mechanisms at what are called cholinergic synapses. It is also clear that in nervous tissue as in other biochemical systems the effects of purine transmitters are closely linked with cellular biochemical processes such as the

formation of cyclic AMP within cells³⁴.

In the past few months, understandably, there has been a great deal of excitement at the opportunities for investigation provided by the apparent discovery of an entirely new class of neurotransmitters. There is great interest in the search for materials analogous to the simple purines that may bind to the receptors but also an interest in the effects of more complicated purine substances on nervous tissue.

The probable consequences of this development, following very closely on the heels of excitement about neuropeptides, is likely to be that far-seeing neurobiologists will in future require that claims that this or that substance may function as a neurotransmitter should be backed up not merely by a demonstration that there are entities within the cell membrane which they will bind, and by circumstantial evidence that only some parts of the nervous system contain appropriate receptors, but also by evidence that a single molecule of such a material, bound to its alleged receptor, will indeed produce some definable physiological effect in the cell concerned. There is, in other words, a suspicion that the currency of neurotransmitters is being revalued by the discovery of too many of them. □

Neuronal plasticity, learning and remembering

THE phenomena of learning and memory imply that it must be possible for nervous systems to acquire permanently the feature that is aptly described as a cast of mind. What can be the molecular or cellular correlates? In the past 30 years, several devices by means of which memories, or learned behaviour, might be encoded within cells have been suggested. The synthesis of proteins or nucleic acid molecules embodying pertinent information have, for example, been canvassed as potential repositories of memory and learning.

Most of the attempts to sustain such hypotheses have, however, foundered on experimental difficulties. In any case, there is no obvious biochemical mechanism whereby information stored in the sequences of proteins or nucleic acid molecules could quickly be recovered, or serve as a continuing influence on the behaviour of a neurone.

Fortunately, however, there are more direct mechanisms by which neurones may acquire long-term characteristics, linked with the mechanisms by which the flux of ions through the cell membrane is regulated. Thus the work of Kandel and his associates³⁷ on the behaviour of two sets of neurones from the abdominal ganglia of the marine snail *Aplysia* has now provided a

good working model of the mechanisms of elementary learning processes.

The key to the understanding of long-term changes in the behaviour of these neuronal cells appears to be the accumulation of Ca^{2+} ions in the presynaptic cells. The more recent work thus derives from the observation of del Castillo and Katz²⁷ that Ca^{2+} ions are necessary for the extrusion of a vesicle filled with neurotransmitter at a presynaptic junction. The long-term changes now proposed as the basis of learning and memory involve changes in the presynaptic cell which affect the effectiveness of the synaptic junction.

Modes of learning

In simple animals such as *Aplysia* but also (on reflection) in higher organisms, two distinct modes of learning appear to play an elementary role — the processes of habituation and of sensitization, by which organisms adapt either so as to ignore an external stimulus repeatedly presented or to respond sensitively to normally innocuous stimuli if these are associated with other noxious stimuli (typified by Pavlov's dogs' behaviour).

Two parallel mechanisms for rudimentary learning of this kind have been identified by Kandel's work in neurones of

Even chemists can do something

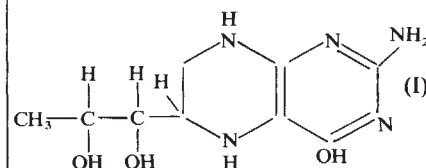
If the neurosciences are as interdisciplinary as claimed, there should be room for everybody. The contributions that electrical engineers have made (and still can make) to the techniques of electrophysiology are clear but what can, say, organic chemists contribute? Here is a nice example, from the Laboratory of Neurochemistry at the National Institute of Mental Health of the US National Institutes of Health.

Phenylketonuria is a genetic disease consisting of the failure of those who inherit the (recessive) condition to convert phenylalanine to tyrosine, an essential amino acid. Although this defect can be made good by a diet supplemented by tyrosine, the most distressing symptom of the untreated disease, severe mental defect, is not always avoided.

The transformation of phenylalanine to tyrosine involves merely the enzymatic removal of a hydroxyl group from a molecule of phenylalanine, in which one molecule of a coenzyme called tetrahydrobiopterin is chemically reduced. A second enzyme is needed to restore the reduced form to its original condition.

Defects of the production of either enzyme could be responsible for genetically acquired phenylketonuria, as could in principle a defect in the production of the coenzyme. So may it not be that those patients acquiring the disease in whom mental defects (and other neurological symptoms) persist in spite of a well-controlled diet may suffer from a lack of the coenzyme.

Clinical evidence³⁵ that this may be the case has been provided by a study of the relationship between the concentrations of the coenzyme and of phenylalanine in the blood of patients resistant to dietary treatment. In the circumstances, the obvious therapy would be the administration of the coenzyme, which has been shown to be capable of crossing the barrier between the blood and the brain.



Indeed, by chemical synthesis, Dr Seymour Kaufman and his group³⁶ have shown that a methyl derivative of the coenzyme may be even more effective, if only because it is likely to persist for longer in the metabolic environment of the brain. This compound (I), 6-methyltetrahydrobiopterin, is now being tried with three young patients whose response to a tyrosine diet has been less than satisfactory. □

Aplysia. Either single presynaptic neurones can be modified or changes may be brought about at presynaptic junctions fed by connections from other cells.

But how can the past history of a neurone affect its present and future behaviour? Classical electrophysiological studies (see, for example, Fatt and Katz¹⁵) had demonstrated the existence of an electrical potential across neuromuscular synapses, which determine the effectiveness of signal transmission. Rudimentary learning processes must affect the magnitude of this potential; since the 1940s, two effects have been recognized — the enhancement of transmission after a rapid series of strong action potentials in the presynaptic cell and, conversely, the depression of transmission after a series of low-frequency potentials in the presynaptic cell (and which are models for sensitization and habituation respectively).

The working hypothesis, which Kandel and his associates have confirmed in the conveniently large neurones of *Aplysia*, is that a rapid series of action potentials in a presynaptic cell, each of which evokes an influx of the Ca^{2+} ions needed for the release of neurotransmitter, leads to an accumulation of Ca^{2+} in the presynaptic space; the ions are removed only slowly by buffering mechanisms in the cell, whence excess calcium is presumably removed by the slow processes of calcium transport. An accumulation of Ca^{2+} in the presynaptic cell is supposed to increase the efficacy of neurotransmitter release, but it has also now been shown that high concentrations of Ca^{2+} within the cell enhance the outward flow of K^{+} across the membrane, thus determining the intensity and duration of the action potential that the presynaptic cell will support (and further enhancing the inward flow of Ca^{2+} in a positive feedback loop).

Perhaps the most striking feature of this recent work is that the influx of Ca^{2+} is regulated not only by the voltage at the presynaptic terminal but by neurotransmitters, by the concentration of cyclic AMP and by the intracellular concentration of Ca^{2+} . The inference is that the proteins serving as Ca^{2+} channels in the cell membrane are regulated by these several influences (and, no doubt, others still to be determined). And, reciprocally, it is inferred that the K^{+} channels are similarly regulated.

Another finding is that, in the neurones of *Aplysia*, the neurotransmitter serotonin, flowing from synaptic junctions with other parallel presynaptic cells, is one of the regulators of Ca^{2+} and K^{+} transmission at these junctions.

The mechanisms of these processes are, as yet, unknown. The notion that ion channels in membranes may be influenced by several chemical regulators (not to mention membrane voltage) implies either a modification or an extension of the classical view that ion channels are of only two types — those which remain open, allowing

the passive leakage of ions, and those which are unambiguously open or shut under the influence of membrane voltage or a chemical transmitter.

Whatever the details, it seems possible by these means to account for Kandel's observation that changes in neuronal behaviour induced by the regulation of Ca^{2+} in presynaptic neurones may persist for periods measured in hours.

The question whether such mechanisms may be a sufficient basis for persistent

behaviour patterns and for long-term memory of the kind of which human beings are capable remains open. There are, however, obvious means by which closed circuits of neurones within a parallel neuronal network could serve to reinforce behavioural changes of the kind now observed in single cells — and, at the same time, account for the distributed nature of memory and learning most dramatically made evident when nervous systems are injured³⁹. □

Improved techniques for brain anatomists

THE peculiar difficulty of the anatomy of the nervous system is twofold — the complexity of the system, especially of the connections between one neurone and others, and the likelihood that apparently similar neurones, even when more or less adjacent, may have different functions. As in the revival of neurophysiology in the

past decade, the foundation of the dramatic advance of knowledge of the anatomy of the central nervous system in that time is a consequence of the development of powerful and sophisticated techniques. And the fruitfulness of these techniques seems widely appreciated, with the result that the search for further refinements engages the attention of a great many of those working in the field.

Three groups of techniques have enlivened the study of the anatomy of the nervous system in the past few years, and respectively make it possible to trace the patterns of complicated nerve cells even within structures such as the cerebral cortex where the shapes and interconnections of the neurones are perhaps more complicated than anywhere; techniques that allow the identification of neurones with some specific property; and techniques for defining the specific functions of individual neurones dynamically, as they occur.

Of all the techniques developed in the past few years for making the pattern of nerve cells apparent, that of injecting or infusing horseradish peroxidase into a neurone is perhaps the most surprising. The principle is that the enzyme is capable of being carried against the normal flow of materials within a cell so that, if incorporated into a neurone at the terminus of an axon, it will be found to fill the whole cell after a period of several hours. (The mechanism of retrograde transport is not understood.) Cells can be made visible by the use of substrate for the enzyme, but are also detectable in both the optical and the electron microscope.

Such techniques have made possible the identification of neurones such as that in Fig. 9 which is a cell in the visual cortex of the cat⁴¹. Such patterns are reconstructed from serial sections of cortical tissue, a procedure that provides a set of co-ordinates for each of the branches of a neurone and which thus makes possible the reconstruction of three-dimensional patterns by the use of computer-driven visual displays. In many physiological

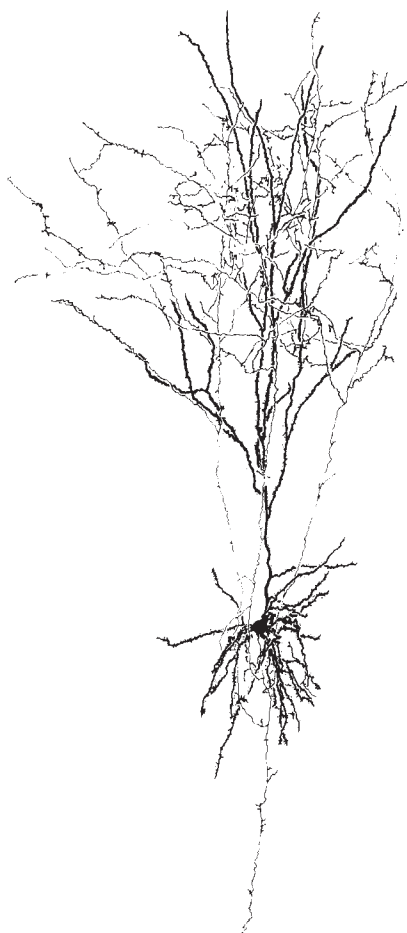


Fig.9 A cell from the visual cortex of the cat (from Gilbert and Wiesel⁴¹). The cell nucleus lies in the lowermost layer of the visual cortex. The upper dendrites lie in or around layer IV, where input from the eyes is first received. The pattern spans a distance of about 1 millimetre.

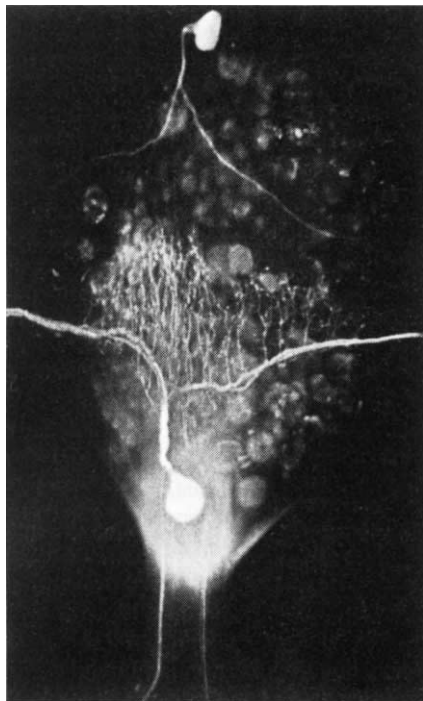


Fig. 10 A photograph showing a Leydig cell in the leech ganglion injected with Lucifer yellow dye. The lower image is the injected cell; the upper, the companion cell to which the first is dicoupled (from Stewart⁴²).

laboratories, making these structures turn round on the surface of a video screen is at least as compelling as the computer game called "Space Invaders".

The use of horseradish peroxidase for such purposes is not, however, straightforward. The snags include the possibility of leakage at points where neurones may be damaged, the need for a careful guess of the time that will be taken for a neurone to be filled with enzyme and the need that the neurone should be incubated in a living animal (which must afterwards be killed).

Of necessity, the horseradish peroxidase will map only single neurones. The surprising observation is that simple amino acids such as glycine will not merely diffuse through cell bodies (in the forward direction in which neuronal signals are usually transmitted) but will also be transferred across synaptic junctions so as to provide, at least in some circumstances, a map of complete neuronal pathways from a sensory input cell in, say, the retina to the appropriate part of the cerebral cortex.

Amino acid transport is made visible by the use of tritiated materials followed by autoradiography. The two techniques can of course be used in conjunction with each other, and have been used with good effect to throw light on the interconnections between different parts of the visual cortex.

In the past few years, similar use has been made of fluorescent dyes, the material called Lucifer yellow in particular⁴². The principle here is that the material (whose molecular weight is relatively small) diffuses rapidly through the cytoplasm of

neurones and may span neural junctions. But the dye can also be coupled covalently to specific proteins so as to define the distribution of known materials within cells. Like horseradish peroxidase, Lucifer yellow is transported in a retrograde direction.

To a large extent, these mapping techniques are complementary; their ranges of application overlap. Plainly there need be no end to the list of neuronal mapping agents in use. Similarly, especially since the development of monoclonal antibodies, there need be no end to the list of materials that can be used for identifying neurones with a certain specificity.

Monoclonal antibodies

For more than a decade, immunofluorescence techniques have been used to map parts of the nervous system rich in particular materials, neurotransmitters such as dopamine for example, but usually provide only scanty information about the degree to which such materials lie inside or outside the cell concerned. Monoclonal antibodies, however, offer the best means of detecting specific components of cell membranes — given that, with luck, a single molecule of some specific cell antigen may be sufficient to yield an indefinite supply of antibody.

So far, most applications of this kind have, however, been effective against somewhat ill-defined antigens, thought to be characteristic of different types of neuronal cells. The search for monoclonal antibodies against, say, particular receptors on the cell surface — necessarily a kind of fishing expedition — is most effective when the receptor concerned can be coupled to some known protein. This is the means by which a monoclonal antibody against the voltage-gated Na^+ channel has been developed and by which a similar material effective against the sodium-potassium pump is likely to be found in the near future.

Perhaps the most remarkable result⁴³ of this procedure so far is the use of a monoclonal antibody against an unknown antigen in the chick retina which suggests that there is a well-defined gradient of the antigen across the visual field. The concentration of the antigen varies by a factor of more than 35 from the centre of the field to the outside, while the material appears to be associated preferentially with the neural cells, and not the light-sensitive cells.

The authors of this research, Trisler, Schneider and Nirenberg, suggest that they may have come across a material which serves to mark the position in the retina appropriate to neurones of different types. In practice, during the development of the retina, layers of cells are added at the periphery so that the material may also be a marker of the stage of development of the animal as a whole. Evidence that the same marker occurs in other neural tissue of the chick has been provided.

This investigation is suggestive of others that might be carried out and which may be particularly relevant to the development of the cerebral cortex. For there, it is now known, neurones migrate towards their "proper" positions in the cortex in the gestation period of primates, apparently finding their proper positions unambiguously. Is it possible that there too a developmental marker may be found?

More recently, the recognition of parts of the nervous system with particular functions has been tackled in a quite different way, by the development (by Dr L. Sokoloff and his associates at the National Institutes of Health) of a technique for telling where metabolic activity is highest⁴⁴. The starting point is the recognition that the metabolism of neurones is supported primarily by glucose and that provision of deoxyglucose (which is not metabolized by neurones) should lead to the accumulation of deoxyglucose in the most active cells.

This prediction, amply confirmed in laboratory studies, has now been turned (by substituting atoms of deoxyglucose by others recognizable by agnostic X rays) into a practical tool of potential clinical importance. Thus, for example, it appears possible to distinguish diagnostically between brain tumours supposed on surgical grounds to be malignant, thus avoiding surgical intervention when this may not be necessary.

More recently, a similar technique for identifying metabolically active parts of the central nervous system, but based either on the rate of protein synthesis or of that of messenger RNA, has been added to the Sokoloff repertoire (see for example ref. 45). Figure 12 shows the concentration of ^{14}C -leucine in the lateral geniculate nuclei (the relay stations for visual signals

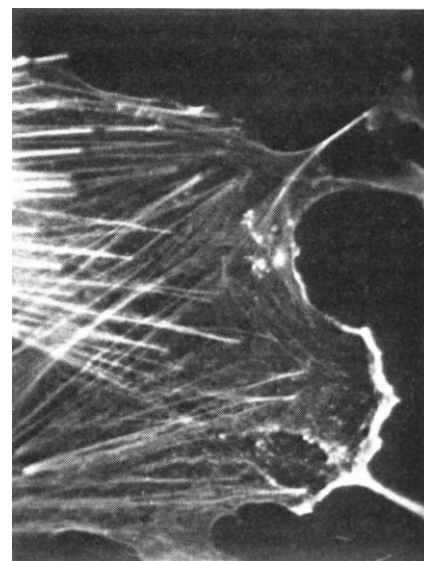


Fig. 11 The use of Lucifer yellow dye (in this case coupled with anti-actin antibody) to demonstrate intracellular structure, in this case actin filaments in human lung cells (from Stewart⁴²).

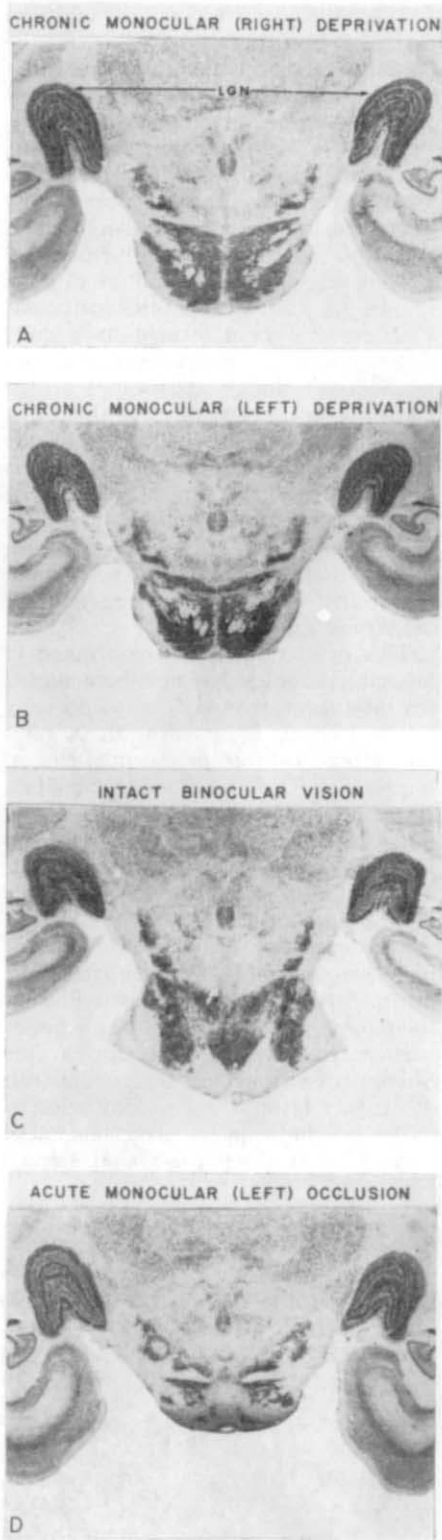


Fig. 12 Autoradiographs of sections of monkey brain through the lateral geniculate nuclei, using ^{14}C -leucine. The geniculate nuclei are the horseshoe-shaped structures: A, from a monkey with intact binocular vision; B, from one whose left eye had been blocked up for a short period; C, from a monkey whose right eye had been blocked up from the 2nd to 25th days of life; D, the same as (C) but with the left eye occluded. Comparison of (C) and (D) shows that the pattern of activity in the two pairs of nuclei is inverted (from Kennedy *et al.*⁴⁵).

between the retina and the cortex) in a rhesus monkey subjected to various forms of ocular deprivation.

For the rest, the technique of electrical recording from living neuronal tissue, the basis of the demonstration in the past decade that, for example, particular structures in the visual cortex are concerned with the processing of

information bearing on visual information of a particular kind — the orientation of edges or lines, for example — continues. The procedures are, of course, time consuming and require great skill, surgical and physiological. But in identifying the function of particular structures in the central nervous system and especially in the brain, there is no substitute for them. [1]

Cortical questions not yet answered

HISTORICALLY, the anatomy of the brain has been through several ups and downs. In the old days, when the phrenologists confidently named parts of the skull with particular relevance to mental functions, it seemed to be generally accepted that an organ such as the brain should allocate different functions to different parts of itself. But with the decline of phrenology and superstition, and the recognition that the brain can be damaged severely and yet to some extent recover, the anatomical approach to brain function ceased to be fashionable.

More recently, with the accumulation of anatomical knowledge of the structure of the vertebrate nervous system, the pendulum has swung again. There are indeed parts of the brain concerned with the processing of sensory information of different kinds. But even on the macroscopic scale on which patches of neuronal tissue several millimetres across may be correlated with the processing of specific kinds of information, it has become clear that parallel processing is commonplace.

Functional correlation

The obvious approach to the relationship between the functioning of the brain as such and the functioning of the neurones of which it is principally composed is to look for patterns of connection between adjacent neurones that may help to throw light on the relationship between microscopic and macroscopic phenomena.

Unfortunately, the only part of the human (and primate) brain in which such an approach might be feasible is the cerebellum, where the arrangement of neurones in layers (lamellae) and their connections with each other are simple enough to suggest that function might indeed be related to structure; but although the cerebellum has been shown to be linked with motor activity, the mental function of the cerebellum remains obscure.

Inevitably, however, in the past two decades, attention has centred on the cerebral cortex both in vertebrates and primates, principally because of the evidence that has accumulated that the cortex (a layer of grey matter two millimetres thick with a characteristic

pattern of folding in each species) is one of the first recipients of sensory information. By now, a great deal has been done to describe the structure of the cortex, and of the cells that it contains, in those areas concerned with the processing of sensory information from the surface of the body and the processing of information collected by the retinae in the visual system; but less is known about the parts of the cortex concerned with auditory and olfactory information.

The cortex

What follows is not a description of the structure of the cortex, as elaborated in the past few years, but rather a list of some of the generalizations that may be tenable and of the conceptual problems that they approach.

The surface of the cortex in primates has now been divided into patches concerned with the processing of different kinds of information, usually on the basis of electrophysiological recordings and the backward tracing of neuronal pathways. Both in the visual system and the somatosensory system, the surface of the cortex appears to represent a kind of topographic map of the outside world as perceived by the two senses concerned. But in the visual system the area to which sensory signals are first relayed lies immediately alongside at least three others in which topographic maps of the outside world are presented.

Why should this be? One inference is⁴⁶ that the adjacent topographical maps of the outside world as perceived visually are concerned with the perception of different aspects of the visual scene. So much has now been substantially confirmed. The primary area in the visual cortex, that to which visual signals are fed most directly, appears to be primarily concerned with the measurement of orientation (of edges and lines). On the other hand, the cortical area responsible for colour perception (whose effectiveness in this respect has been confirmed by a demonstration that monkeys damaged in such a way are colour blind) contains neurones which do not respond to lines of particular orientation nor to movement across the visual field⁴⁷.

Zeki has argued that such an arrangement is necessary because, for example, the perception of colour requires not merely an analysis by the visual system of the "colour" of light arriving at the retina from a particular point in the visual field, but also a knowledge (reflected in the responses of neurones) of the intensity of light from other surrounding areas⁴⁸.

Inevitably, such an arrangement also ensures the advantages of parallel processing in the cortex — one area may be made inactive without the whole sense of vision being lost. Even so, this particular arrangement of topographic maps of the outside world, identified at least in the visual and somatosensory systems, leaves a number of questions unanswered. Why, for example, are the first and second of these topographic maps never images of each other? How has the elaboration of areas of cortex concerned with specialized aspects of vision occurred in the course of evolution? (It goes without saying that there is hardly any direct evidence of the arrangement of cortical areas in the visual system of the human being.) And, in any case, where in the brain does some neurone or group of neurones behave in a way equivalent to saying "That's a motor car"?

Neuronal arrangement

A second common feature of the structure of the areas of the cerebral cortex is that neurones appear to be arranged in layers parallel to the (convoluted) external surfaces. In the visual cortex of the primate, cells in what is conventionally called layer IV seem predominantly concerned with receiving signals from the lateral geniculate nucleus. The two layers above that appear (from studies typified by that of Fig.12) to be concerned with transfer of the messages to other cortical areas, while the lower (deeper) layers appear primarily to be concerned with relaying messages to deeper structures within the brain. The outermost layer of the visual cortex is not even well guessed at.

One of the difficulties that now need to

be resolved is the conceptual gap that has opened up between the anatomical description of the connections between these several layers of the cortex and the notion of what their different functions may mean. It is, of course, conceivable that the explanation may be that different layers of the cortex are concerned with different combinations of aspects of sensory perception. But the difficulty has not been resolved.

The grey matter

The third feature of the gross anatomy of the cerebral cortex, as so far defined, is that even within areas of grey matter known to be concerned with one aspect of one sensory perception (the primary area of visual perception, for example), neurones are arranged in a way that betokens specialization. Thus in the primary visual cortex (conventionally known as area 17), the grey matter appears to be arranged in columns, with adjacent columns apparently receiving inputs primarily from one eye or the other. Similar columnar structures have been recognized in parts of the cortex concerned with other senses, the somatosensory system for example. It is by no means clear what function this arrangement may perform nor what its presumed advantages may be.

Fourth, all sensory systems in the cortex so far described appear to entail the relaying of signals from peripheral sensory elements (rods and cones in the retina, nerve endings in the skin) to the appropriate parts of the cortex by means of intermediate staging posts, the geniculate nucleus in the visual system for example. Teleologically speaking, such an arrangement has the advantage that an external sensory element is not entirely committed to one part of the cortex for the correct processing of the information that it may be able to collect. In practice, the same intermediate nuclei may have the function of being able to arrange for monitory connections between different sensory systems before the information

that any one collects is subjected to the full panoply of information processing. But the question remains of how this self-checking system has emerged in the course of primate evolution.

Fifth, there is the problem of feedback in the visual system. While most neurones connecting the retina to the two lateral geniculate nuclei through the optic chiasm transfer signals upwards or inwards, the anatomists have identified neuronal connections between the geniculate nuclei and the retinae that work in the opposite direction. Similarly, the primary visual cortex appears to feed back to the geniculate nuclei.

The auditory system

Even richer feedback loops have been identified anatomically in the auditory system. The obvious question is whether their function is to adjust the input of sensory elements to the "needs" of the entire perceptual system. The trouble with that assumption is that it implies not merely that the visual cortex is a representation of the seen outside world, and that other areas of the cortex similarly represent the sensations of other senses, but that hidden somewhere in the middle is a homunculus, our representation of ourselves.

It may well be the case that the difficulties so far encountered in relating neural anatomy to nervous function stem not so much from the complexity of the anatomical investigations but from the lack of guiding principles for the anatomists. Zeki's arguments about the requirements of effective colour vision, while largely theoretical at least as far as the sheer anatomy of the nervous system is concerned, must surely rank as hypotheses in need of testing. (The fact that they may be substantiated by experiment as well is not finally clinching.) Thus the time may have come when the neuroscientists should take time off to consider questions such as the cognitive nature of the functions they look for in their cross-sections through the cortex. □

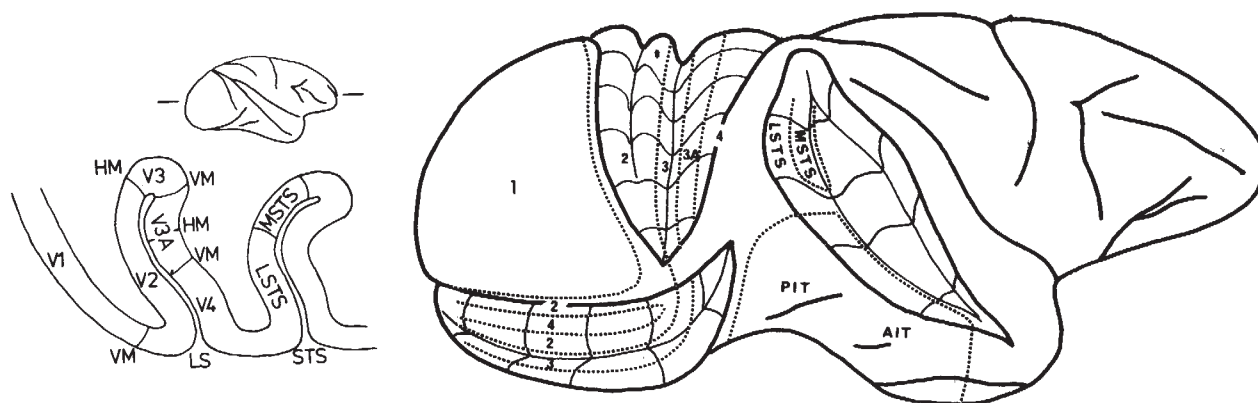


Fig.13 Diagrams showing the arrangement of the visual cortex in the rhesus monkey. The bottom left-hand diagram is a horizontal section through the back half of the right hemisphere. V1 is the primary visual cortex (also known as area 17). V2 carries a retinotopic representation which is a mirror image (through the plane of VM) of that in V1. The other visual areas reading from right to left along the cortex are V3, V3A, V4 and LSTS and MSTs, the lateral and medial visual areas in the brain cleft known as the superior temporal sulcus.

Thinking seriously about thinking

GRAND thoughts about the nature of perception or of consciousness are necessary and, in any case, irrepressible. What the neurosciences are most in need of, however, are serious-minded attempts to define what the central nervous system must accomplish to perform the cognitive tasks we know we are capable of. The late Dr David Marr was probably the first to take such an agenda seriously².

The problem on which he embarked, in 1975, was that of binocular vision. The problem was well chosen — there was a chance of specifying what it amounted to. The underlying issue is that of how it may be possible, given that the retinae of the eyes each present a two-dimensional representation of the outside world to the inputs to the optic nerves, that the brain as a whole is able to perceive the world in three dimensions. The simple answer is that there are two eyes, so that stereopsis comes into play.

Stereopsis

Stereopsis implies that the nervous system must have some means of comparing the output from two retinae in as much detail as may be necessary. The anatomical site for the exchange of information is well known — the optic chiasm, where the two optic nerves cross over. The further implication, however, is that at that point, the subtraction of the sensations (signal strengths) from equivalent points in the visual field are as important as their sum.

Indeed, the sentence with which Marr and Nishihara began their publication of 1978 (ref. 49) is as valid now as then:

Modern neurophysiology has learned much about the operation of the individual nerve cell, but unpleasantly little about the meaning of the circuits they compose in the brain. The reasons for this can be attributed, at least in part, to a failure to recognise what it means to understand a complex information-processing system, for a complex system cannot be understood as a simple extrapolation from the properties of its elementary components.

The issue, the argument continued, is that of deciding at what level of complexity and aggregation the phenomena should be described.

The difficulty, as always in these circumstances, is that of converting precept into practice. It would be folly to suggest that Marr's specification of algorithms by which a sense of binocular vision might be reconstructed from the two-dimensional representation of the visual field provided by the two retinae is perfect, although it has served as a pointer

for psychological experiments from which one algorithm has emerged as a more probable candidate for the truth than others.

One essential feature of any successful process of computation of visual depth, however, must be that visual signals from the two retinae must be accessible to the part of the central nervous system where the computation is carried out. Another is that the task cannot be accomplished without comparative information from different parts of the visual field. Thus the simplest retinotopic map of the outside world, that apparently provided in the primary area of the visual cortex, will not suffice for binocular vision.

The nature of the task is well illustrated by Necker's illusion (Fig. 14). The first panel is a geometrical representation of a cube, the cognitive generalization of the cube that we hold in our heads (perhaps with some help from mathematics teachers). The other two panels are more accurate representations of what may be represented by the topographic maps in the primary visual cortex by the sight of a cube. Distinguishing between the representations in the second and third panels requires a sense of depth. Knowing that the object concerned is a cube (when, of course, that may be a trick) requires some other faculty.

Marr's later argument (with Nishihara, see ref. 49) about the cognitive tasks that must be accomplished before the three-dimensional shape on an object can be comprehended are interesting not merely for their insight but also for the points at which they break down.

Cognitive tasks

Marr did not dodge the task of specifying the levels of generalization at which cognitive tasks must be specified. Broadly speaking, there are four. First, describe the elements of the network, describe the properties of the neurone, for example. Second, figure out how circuit elements such as these might serve the purposes of arithmetic — adding, subtracting, differentiating, performing Fourier analysis or what have you. Third, construct an algorithm that will ensure that the product of the computation is *some* three-dimensional structure, for which purpose any of the three panels of Fig. 14 would suffice. Finally, develop a theory of the process that will enable the homunculus inside the cortex to conclude that the object seen (taking past experience and all other relevant information into account) is most probably a cube.

The inference is that, for the time being, the neurosciences are placed somewhere between the first and second levels of this

programme, but also that the human cognitive function includes all four levels of abstraction.

Marr's theory of how the central nervous system constructs three-dimensional images of what the objects outside the eyes are like is nevertheless far from persuasive. Part of the difficulty is that the argument concerns the visual system in isolation from other parts of the central nervous system, not merely those receiving sensory information but those concerned with what we know (that is, have learned) about the world we live in.

Thus the argument, concerned as much with the construction of an algorithm for three-dimensional perception as with building a theory of the process, supposes that people engaged on visualizing three-dimensional objects will seek out some

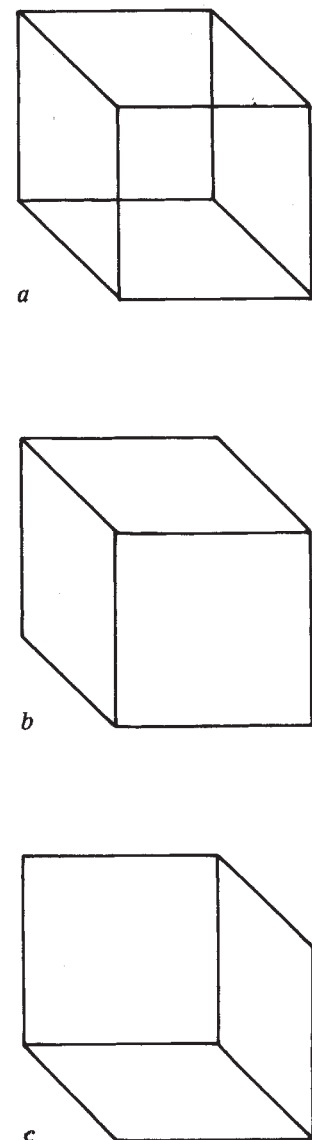


Fig. 14 Necker's illusion. *a* Is a two-dimensional representation of a cube; *b* and *c* are alternative representations of it in three dimensions between which the mind hesitates.

Vision machine

IN an upper window at the Artificial Intelligence Laboratory at the Massachusetts Institute of Technology, a peculiar camera looks out on the main street connecting the two universities in the City of Cambridge. Representations of the camera's visual field are regenerated at intervals of about a second — a technical limitation, caused by the decision to use simply a linear array of gallium arsenide photodetectors as a means of generating a two-dimensional picture but also by the capacity of the computer. The owners of the camera, however, claim that it is the first true electromechanical vision system.

But what is wrong with an ordinary television camera, which can produce an image of the scene in front of it with much greater speed? The answer is that a television camera (with accompanying video display) is not a replica of a visual system, but an analogue thereof. There is no means, as in the visual system of the primate, by which information subsidiary to the construction of a two-dimensional image can be extracted. Vision in depth is not possible. And there is no way in which some kind of homunculus can be enabled to monitor what is happening.

In the machine the video picture is regenerated from the top, and each sweep across the frame shows that the cars waiting in line to cross the Charles River have moved on a yard or so. The only intermediate use of the explicit representation of visual perception that the system is so far equipped to exploit is to degrade the quality of the image, lumping together the signals from groups of sixteen or so picture elements to form a blurry picture. The resulting images are blurred. But shapes are surprisingly recognizable.

The plan is that there should soon be a second camera, so that it should then be possible to try out the algorithm for binocular vision. It may then be possible also more stringently to test the underlying theory of vision embodied in the computer program — that what matters, in the perception of the outside world, is the definition of the lines (outlines) that mark the one-dimensional contours in a scene where contrasts between light and shade are greatest. The constructors of the camera confess, however, to one conceptual failure. The project is financed partly by the military services in the United States, which have an obvious interest in machines that might be able to see as people do. So could the system be used for spotting tanks on a battlefield, or missiles in their silos? Well, of course. Certainly such a system, if the theory is correct, could recognize such objects. But could it give them a name? Not yet — maybe that requires "higher", cognitive functions.

principal and more or less symmetric axis to which the details of the image may be related. At the level of the algorithm, the suggestion makes sense. It is, however, unpersuasive. It may serve well enough in the identification of a knitting needle or even a human face, but what about a landscape? Or a familiar face in profile? Somehow, the algorithmically derived theory does not ring true.

Implicit recognition of this failure seems to have driven the devotees of Marr's programme to controlled despair⁵⁰. In the past few years, the artificial intelligence

community has slipped into the habit of asserting that the only proof that perception and cognition have been understood is that they (or somebody) can construct a machine that will replicate the process. The strategy is sensible enough: if a machine that will replicate the process of human vision could be built, a demonstration of its power would persuade all kinds of people that the problem of vision had been tackled seriously. Sceptics will, however, complain that simulation is not the same as understanding. □

A few loose ends

THE general impressions created by the neurosciences and their practitioners on the rest of the professional community is that it can be only a matter of time, a few months or at the most a few years, before big questions are finally decided, before the pot of gold is finally disinterred from beneath the rainbow's end. Naturally, the practitioners are unabashed when it turns out that the prudent direction of the hunt has changed. For it will be only a few months, or at most a few years. . . .

This impressionistic survey of what is happening in the neurosciences should give the lie to that always false expectation. Perhaps the most striking of the impressions gathered is that quite old-fashioned approaches to the working of the nervous system appear still to have a great deal of promise left in them.

Is there, perhaps, a case for asking that those about to embark on the isolation and cloning of this or that neuronal gene should first be asked to carry out a simple experiment with the giant axon of the squid, if only to acquire a sense of what neuronal transmission is about? And an understanding of what makes it so interesting as a phenomenon? As things are, there is a danger that the neurosciences will be imprisoned by the fashions from time to time inflicted on them.

It also emerges that several long-standing problems in biology (not much discussed above) and apparently central to the function of a self-respecting nervous system have been skipped over in the headlong rush of the past few years. Here is a brief list:

Circadian rhythm

Although the phenomenon of diurnal metabolic activity is not well attested in human beings, it does appear that insects such as the locust can keep time with the Sun (and often have no choice). Several suggestions have been put forward as to how this might be arranged. Groups of cells linked synaptically together might just do the trick, especially with the entraining help of a diurnal stimulus. But which are those groups of cells, in some insect or

other species? And how precisely do they function?

Schizophrenia

For several years, the text-books have rightly drawn attention to the connection between the most serious (if not necessarily the most common) of psychiatric disabilities and abnormalities of the behaviour of dopamine (a neurotransmitter) in the human central nervous system, especially in the limbic system and in the structure known as the substantia nigra. Once (ten years ago) the case seemed simple. Drugs known to bind to dopamine receptors, the phenothiazines in particular, were found to help alleviate the symptoms of the disease. So what more natural than to suppose that the underlying biochemical defect in schizophrenia is an excess of dopamine by dopaminergic neurones, an increased frequency of dopamine receptors in postsynaptic cells or an increased sensitivity on the part of a normal neurone population?

Unfortunately it has not been possible to make theories of this kind accord with what is known of the disease and its drug treatment without postulating an uncomfortable variety of dopamine receptors, at least one of which is a kind of receptor not known elsewhere — an auto-receptor on the presynaptic neurone which is sensitive to the neurone's own product. Given the social importance of the disease, would not an investigation of the nature of the dopamine receptor (what, physiologically, does its activation do to a cell?) be worthwhile?

Manic-depressive illness

This is a simple question. Why is lithium effective in the treatment of manic depressive illness, a disease in its own way as important as schizophrenia? Presumably lithium has something to do with ion channels. Nobody seems to know what the answer is.

Colour vision

Thomas Young in the seventeenth century

was the first to conclude (theoretically) that the eye must contain receptors for three primary colours. The theory has, of course, been brilliantly confirmed — there are three classes of cones with different spectral sensitivities. Yet Young's argument is now known to be false.

Curiously, the cells of the colour-sensitive visual cortex in the monkey (the fourth visually sensitive area counting from the primary area) respond over comparatively narrow ranges of the spectrum to colours not represented in Young's list of primary colours or by the colour-sensitive pigments in the cones of the retina. Moreover, from common experience, it is known that the perception of colour remains constant over an enormous range of visual intensity. Also, Land's two-colour theory of colour vision (in which Young's third colour is replaced by the third dimension of intensity) appears to fit the facts quite well. Zeki^{47,48} has defined the problem and resolved some parts of it. What can be said about the remaining conundrums and their bearing on the rest of mental function?

Neuronal peptides

The fuss in the past five years about neuronal peptides (deserving, as a social phenomenon and investigation of its own) must mean something, for there is never smoke without fire. But what? The enkephalins appear to bind to the opiate receptors (whose physiological function has not yet been defined). But do the other peptides (with the possible exception of substance P) have a neuronal function? If not, should an understanding of their function in mammalian brain be sought in the mechanisms of the secretion of hypothalamic hormones, a question not given much attention?

Auditory system

What is to be made of the mechanism of perception of sound by the mammalian ear? Sensory transduction is markedly different from that of other sensory inputs, consisting of a mechanism whereby mechanical pressure fluctuations within the ear are translated into neuronal signals as a result of the specialized behaviour of hair cells in the cochlea. Although the hair cells are generally arranged along the membrane within the cochlea according to their frequency response, it is by no means clear what representations of these sounds is eventually conveyed to the part of the cortex (at the outward fold known as the superior temporal gyrus) representing the outward impression of the ears. Nor is it clear why the ascending nerves of the auditory system pass through no fewer than four intermediate nuclei before reaching the cortex, from each of which there is a rich supply of nerves running in the opposite direction. Could this complicated arrangement be an evolutionary hangover from more primitive times?

Smell

What is it, in molecular terms, that the olfactory system responds to? Shape? Vibration frequency? Or something else?

Acuity

Psychological measurements of the sensitivity of the senses usually conclude that, in favourable circumstances, even the human senses are capable of surprisingly accurate measurement. Ears in good condition can, for example, detect frequencies of the pitch of sounds heard independently that differ by no more than a few cycles a second. Is the basis of this operation some absolute set of standards or, alternatively, some physical principle such as the production of a beat frequency with a set of standard local oscillators?

In the visual system, the relatively high performance of the human eye (sensitive to changes of position of a few minutes of arc in the visual field under the best conditions) is well accounted for by the density of neurones in the primary visual cortex. The sensitive parts of the somato-sensory system appear to owe their acuity to the way in which they are overrepresented in the cortical maps of the outside world, as perceived by the peripheral nervous system. But can this general principle be confirmed for other kinds of perception?

Development

The description, in the past few years, of the migration to the cortex of neurones within the developing mammalian embryo is nevertheless a reminder that the mammalian nervous system presents curious problems in developmental biology. The general rule is that the simpler an organism, the more predetermined ('hardwired') is its endowment of neurones. By what mechanisms are these wiring systems established? Yet even the simplest forms of organisms (*Aplysia*, for example) are capable of plasticity.

What mechanisms account for residual post natal plasticity in higher organisms? Modification of membrane transmission in networks of cells? The growth or atrophy of neuronal processes? Or something quite different? What, in any case, is the function of the elusive nerve growth factor, whose role in neuronal cell biology seems to become less well defined as its chemical structure is better known?

These, it may be thought, are fields of investigation well within the reach of the techniques which have sustained neurobiology in the past two decades.

These and others are, of course, fields of investigation at the centre of many people's interests. Some of them are well within the reach of the techniques that have sustained neurobiology in the past two decades. Others, the problem of the development of the nervous system for example, will probably require the development of new techniques — the procedures so far used, fruitful though they have been, are un-

happily time-consuming and frustrating.

The question of how the brain as a whole works, no doubt unfairly referred to as a subject for retired gentlemen, is nevertheless at this stage unlikely to be a fruitful stimulus for the further understanding of how the nervous system functions. This does not, however, mean that there is no place in contemporary neurobiology for theoreticians. On the contrary, as the work of David Marr has amply demonstrated, there is a great need for tangible models of specific parts of neural mechanisms.

The value of these theoretical approaches is nicely illustrated by the argument due to Crick, Marr and Poggio⁵¹ on the acuity of visual perception. Marr's approach to the theory of how an abstract image of the external visual world is constructed relied largely on the assumption that one of the chief objectives of visual processing is to determine the places at which the outlines of objects can be discerned, perhaps by the rate at which the intensity of light changes over the visual field.

This is also the underlying principle of the artificial vision system developed at MIT (see p. 531): that the visual field has a million picture elements and the computer processing consists of comparing the intensities of the picture elements in such a way as to define the outlines of objects 'seen'.

Both the development of a computer program capable of operating within the limitations of the hardware available and the mathematical nature of the problem have required the investigation of problems in information theory that are themselves of great interest.

What Crick, Marr and Poggio have shown is that it is indeed possible for a system such as the human retina and the associated visual cortex, with primary visual receptors separated from each other by angular distances of 25 minutes of arc, to detect irregularities in the positioning of points of light which are themselves an order of magnitude less. That this is possible turns on arguments about the way in which the general shape of a mathematical function can be reconstructed from a sample of its values taken on some regular grid of coordinates — and on the assumption that the differential coefficients of the function are not too large. Such arguments promise to be able to build bridges between the understanding of the mechanism of perception in neuronal terms and the psychometric laboratory.

But may there not be scope for other kinds of theoretical arguments at a still more rudimentary level? If, for example, the typical neurone in a nervous system contains several types of channels for the transfer of ions across the membrane, and if some of these channels are sensitive to other extracellular influences or even to the ions themselves, a complicated but nevertheless soluble problem has been defined. It may be more productive to tackle that first, before going on to consider the mathematics of consciousness. □

Problems of organization

THE neurosciences are proud to call themselves an interdisciplinary gathering of people. But neuroscientists themselves often overlook the extent over which their component disciplines are spread. The field has its roots in clinical medicine. Physiology entered the scene only relatively recently, perhaps a century ago. But now, all kinds of disciplines have a finger in the pie, straightforward chemists and information scientists for example.

Growth

The neuroscientists also often forget how quickly their field has grown in the past decade or so, and how in the process it has been transformed. Until the mid-1950s or thereabouts, neuroscientists were typically people working in small laboratories, pursuing particular investigations in the time they had to spare from their other duties in the classroom and the committee room. The notion that there might be as much scope, in the understanding of how nervous systems work, for a transformation of professional opinion as there had been in molecular biology in the early 1950s, was reluctantly accepted by the scientific community and by the grant-making agencies. It is also fair to say that even at this late stage, many practitioners regret that their field of interest has become the excited (and excitable) community that it now is.

Thus the American Society for Neuro-

science now boasts of 7,000 members. Many universities and other academic institutions now boast of an institute of neuroscience. (Johns Hopkins University and University College London have thus been blessed in the past year.) The standard claim on public attention (and the grant-making agencies) is that more neuroscience will in due course mean less psychiatric illness, less drug addiction and possibly even an amelioration of mental defect.

Problems

Two problems arise. First, the neurosciences are probably by their nature destined to remain interdisciplinary. Intellectually, what this means is that students and postdoctoral fellows, trained perhaps as neurosurgeons, biochemists, molecular biologists and physiologists, will continue to provide the energy with which the understanding of nervous function is deepened. Of necessity, these recruits to what are called the neurosciences retain their affiliations and their skills. And there is no sense in the reasoning that the barriers between them can be broken down by renaming university departments responsible for undergraduate teaching.

Second, given the nature of the problem, the neurosciences are likely always to require that practitioners should work with specialized techniques in relatively narrow fields. Umbrella organizations, either the American Society for Neuroscience or

Professor F.O. Schmitt's personal creation, the Neurosciences Study Program, have a useful role to play in providing forums for discussion, but their influence is necessarily modest.

Communication

So may it be that what the field is most in need of is not more money or more people but a more durable method of communication than those with which it is now provided? Chemists, after all, rarely read the literature of the clinical sciences. Information scientists are attracted to problems of the nervous system only accidentally, when they happen to come across some reference in von Neumann, Shannon or even Schrödinger. Inevitably, the trouble is that such a vehicle for communication could not be a formal scientific journal; it would have to be, instead, a way of making people confront each other's problems.

Reconciliation

There are also geographical or cultural differences to be reconciled. Europeans like to think that they have invented most of the techniques that matter (but see ref. 44), and that Americans have jumped on the bandwagons thus set in motion; Americans, on the other hand, rightly suppose that they have made the only effective use of the tools at their disposal if, as seems likely, the pace of growth of employment in the neurosciences is likely to be less in future than in the past, can the machinery of communication remain as chauvinistic as it is? □

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Who goes where from here?

This survey of the present state of neurosciences is necessarily a kind of snapshot, one taken from an idiosyncratic point of view. Others might well have paid more attention to other aspects of the functioning of typical nervous systems — the biochemistry of the brain, the psychological tests that can be made of the functioning of these systems and insight that may be gleaned from the way in which typical nervous systems respond to injury, either accidental or deliberate (as in surgery). Much the same conclusion would no doubt emerge from a survey of some other field — high-energy physics for example. The most vivid impressions are those conveyed most forcefully by those accidentally encountered. Nature is grateful to those who have been good enough patiently to answer the questions importunately put to them.

A few generalizations are appropriate. First, the present ferment in the neurosciences is not new. What has happened in the past few years is merely that the community of the neuroscientists has grown, perhaps by an order of magnitude in ten years. Second, the kinds of problems with which people now contend are not very different from those of the 1950s, or for that matter of the 1930s. As always, the most interesting problems are those which cannot unambiguously be resolved for lack of data.

Third, however, a good deal of water has now run under the bridge. Several features of the typical nervous system and even of the human brain are well established. The chances are good that the cooperative functioning of aggregations of neurones will indeed account for the way in which brains of all degrees of complexity perform. One point that needs urgently to be established is whether the behaviour of such a co-operative aggregate will indeed be sharper, less fuzzy at the edges, than the behaviour of single neurones. That must surely be a challenge for the physicists and those in artificial intelligence.

There is also a need for a better understanding of the metabolism of neuronal cells. Some specific interactions between those materials unambiguously known to be neurotransmitters and the downstream cells are reasonably well understood. But by what mechanisms is the replenishment of neuronal transmitters in the presynaptic cell regulated? And what balance

can be struck between the effects of neurotransmitters at synaptic junctions and the more generalized effects of neuroactive materials in the intercellular medium? Some indication of the importance of these metabolic questions may be gleaned from the fact that no less than 14 per cent of the energy consumption of the adult human being is expended on the metabolism of the human brain.

Other issues arise in the anatomical description of real nervous systems. The general tendency is for there to be a more or less geometrical representation of the outside world on the surface of the cortex, in the visual and in other sensory systems, but there is at present only the sketchiest understanding of how such representations may be interconnected. Practically, however, these interconnections are crucial to proper functioning. One can avoid a visible obstacle in one's path without thinking very hard. Is this a field in which psychology may more rapidly contribute to understanding than plain painstaking anatomy?

The conceptual part of the problem of neurobiology also deserves more attention than it has received. Grand philosophical questions — what is consciousness, and so on, are probably less important than more strictly operational questions — by what means do people learn to assign names to familiar objects, providing themselves with a kind of lexicon for the images formed in the visual cortex? Is Chomsky's view that the structure of language implies a kind of universal grammar reflected in the anatomy of the regions of the brain and, if so, what kinds of connections are implied?

The growth of the American Society for Neuroscience in the past few years is at once a measure of the enthusiasm of people (mostly relatively young) for this absorbing field of enquiry and of the willingness of the grant-making agencies to back that enthusiasm with grants. But the growth cannot continue indefinitely. The society's boast, in recent years, that by the end of the century there will be more neuroscientists than adult citizens in the United States is a poor joke. Has the time come when the neurosciences should work out a strategy for maturity?

ARTICLES

Noble gases in the terrestrial planets

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Abundances of primordial noble gases are lower for Mars than for Earth, but are higher for Venus. The data for Venus are attributed to implantation of solar wind in small preplanetary particles. Results for Mars are explained by escape of gas from planetesimals with radius between 5 and 100 km which form within the first 10^7 yr of the Solar System. Volatile loss is associated with melting caused by short-lived radioisotopes such as ^{26}Al .

OUR knowledge of planetary atmospheres has increased markedly over the past decade, thanks mainly to experiments on the Mariner, Pioneer and Viking missions of the United States and the Venera missions of the Soviet Union. Measurements of noble gases in the atmospheres of Mars and Venus, summarized in Table 1, are particularly puzzling: concentrations of ^{22}Ne , ^{36}Ar , ^{84}Kr and ^{132}Xe were unexpectedly low for Mars¹⁻³; concentrations of ^{20}Ne , ^{22}Ne , ^{36}Ar and ^{38}Ar were very high, in contrast, for Venus⁴⁻⁸.

The noble gases provide invaluable constraints on models for the formation of planets and for the evolution of their atmospheres. It is useful to distinguish from the outset between primordial gases (for example, ^3He , ^4He , ^{20}Ne , ^{36}Ar , ^{38}Ar , ^{84}Kr , ^{132}Xe) present in the original solar nebula and radiogenic gases (including ^4He , ^{40}Ar , ^{129}Xe) produced at least in part by decay of rock forming elements such as U, ^{40}K and ^{129}I . The half life of ^{40}K is 1.3×10^9 yr and the abundance of ^{40}Ar reflects both the initial abundance of potassium and the efficiency for degassing over the age of the planet^{9,10}. Interpretation of data for ^{129}Xe is complicated in that the half life of the precursor ^{129}I , is only 1.6×10^7 yr (refs 11, 12). Thus concentrations of ^{129}Xe and ^{129}I may vary significantly over the period of planetary formation.

Primordial noble gases in meteorites fall into two classes^{13,14}, planetary and solar, with relative abundances as given in Fig. 1. The planetary distribution is thought to arise during condensation¹⁵⁻¹⁸ though it may be modified by subsequent evolution. The solar pattern appears to reflect implantation by the solar wind and is displayed prominently in samples from the lunar surface¹⁹. The atmospheres of the terrestrial planets retain neon, argon, krypton and xenon^{1,20-22}. Helium, in contrast, is lost rapidly to space^{23,24}.

An acceptable model for the terrestrial planets must account for: (1) an abundance of primordial neon and argon in Venus' atmosphere ~ 70 times larger than that for Earth; (2) an abundance of primordial neon and argon in Mars' atmosphere ~ 180 times less than that for Earth; (3) ratios of primordial neon to argon similar for all three planets; (4) occurrence of the planetary pattern for Ne, Ar, Kr and Xe in meteorites and in the atmospheres of Mars and Earth, with a departure for Kr on Venus; (5) abundance ratios $^{129}\text{Xe}/^{132}\text{Xe}$ and $^{40}\text{Ar}/^{36}\text{Ar}$ for Mars higher than for Earth; (6) a ratio $^{20}\text{Ne}/^{22}\text{Ne}$ for Venus similar to solar wind, though higher than for Earth; and (7) a ratio $^{36}\text{Ar}/\text{N}$ for Venus higher than for Earth by a factor of 20. The problem may be summarized as follows. Venus has an unexpectedly high concentration of ^{36}Ar , a ratio $\text{Ne}/^{36}\text{Ar}$ similar to Earth, Mars and the planetary component of meteorites, but a distinctly different value for the ratio $^{20}\text{Ne}/^{22}\text{Ne}$. The concentration of ^{36}Ar in Mars' atmosphere is low and cannot be attributed simply to inefficient degassing.

Previous models

Differences in the abundances of noble gases in the atmospheres of terrestrial planets might arise due to variations in the extent to

which parent solid bodies released their original store of volatiles. The abundance of ^{40}Ar gives information on the rate and manner of degassing^{9,10}. The abundance in the atmosphere of Mars is less than that in the terrestrial or Venus atmospheres by a factor of ~ 10 . If we assume, consistent with available data²⁵⁻²⁷, that K should be similar for all three planets, then the degassing efficiency for Mars should be about one-tenth that for Earth and Venus. These considerations suggest that the total planetary abundance of ^{36}Ar for Mars may be 10 times larger than the atmospheric abundance, 18 times less than the terrestrial value. Our estimate for the martian abundance would be reduced if K were low as suggested by Anders and Owen^{20,26,28}. Table 1 includes estimates for the total abundance of ^{36}Ar in each planet. These data were derived by scaling measured concentrations of ^{36}Ar using observed values of ^{40}Ar .

The noble gas results for Mars and Venus are quite unexpected. Models for condensation in the early solar nebula suggest a pattern opposite to that observed^{15,16,29-31} with concentrations highest at Mars where the nebula was relatively cold, lowest at Venus. Concentrations of ^{36}Ar in meteorites^{14,32} range from undetectable to 3×10^{-9} g per g. One might attribute the abundance of ^{36}Ar in a particular planet to aggregation of materials drawn from different meteorite classes³³. Anders and Owen²⁰ proposed that planetary volatiles could be associated with late accretion of volatile rich material with composition similar to meteorites of class C3V. The hypothesis was introduced to account for the Viking results and is generally consistent with the martian data summarized in Table 1. It fails, however, to account for the more recent information from Venus²⁸. Pollack and Black³⁴, working with the Venus data, took a rather different route. They suggested that noble gases could be equilibrated with grains in the primitive nebula at a rate proportional to ambient pressure. The nebula was assumed to be isothermal between the orbits of Venus and Mars to maintain an invariant pattern in noble gases. The pressure must have declined by four orders of magnitude over this distance to account for the decrease in noble gases indicated in Table 1. This requires a scale height in the primitive nebula 20 times larger than one would calculate assuming a central mass equal to that of the contemporary Sun, a difficulty not explicitly considered by Pollack and Black.

Present model

We propose a model for the noble gas concentrations observed in all three terrestrial planets drawing on data from meteorites and lunar soils. We take the view that the origin of Venus' noble gas must be distinct from that of Earth's, as the isotopic composition of neon is different. The similarity of Venus' $^{20}\text{Ne}/^{36}\text{Ar}$ ratio to the terrestrial value must be considered in this case fortuitous. We suggest that Venus' noble gases are derived mainly from solar wind while the terrestrial and martian components condensed from the nebula with an origin similar to

Table 1 Noble gases

	Atmos. ^{36}Ar (g per g)	Atmos. ^{40}Ar (g per g)	Total $^{36}\text{Ar}^*$ (g per g)	$^{20}\text{Ne}/^{36}\text{Ar}^\dagger$	$^{84}\text{Kr}/^{36}\text{Ar}$	$^{132}\text{Xe}/^{36}\text{Ar}$	$\text{N}/^{36}\text{Ar}$	$^{20}\text{Ne}/^{22}\text{Ne}$	$^{40}\text{Ar}/^{36}\text{Ar}$	$^{129}\text{Xe}/^{132}\text{Xe}$
Venus‡	2.4×10^{-9}	2.7×10^{-9}	2×10^{-8}	0.3 ± 0.2	<0.007	?	2.5×10^3	14_{-3}^{+6}	1.0	?
Earth§	3.5×10^{-11}	1.2×10^{-8}	7×10^{-11}	0.52	0.021	7.5×10^{-4}	5×10^4	9.8	296	0.98
Mars	2.0×10^{-13}	7.0×10^{-10}	8×10^{-12}	0.38	0.032	3×10^{-3}	2×10^6	?	3000	2.5
Sun¶			3×10^{-5}							
Solar wind				35	0.0005	6×10^{-5}	1×10^2	13.3	<1	1.05
Flares				32				7.7		
Lunar fines**			8×10^{-7}							
Meteorites**										
C				0.27	0.012	1×10^{-2}	4×10^6	8		1-3
C3V			5×10^{-10}				3×10^5			
C30			2.5×10^{-9}				6×10^4			
H, L, LL			$0.2-5 \times 10^{-11}$	low	0.01	1×10^{-2}	4×10^6			
E			$0.1-1 \times 10^{-9}$							
Gas-rich			$0.02-2 \times 10^{-9}$	20	0.001	low		12.5		
Neon-E								<0.1		
Cosmic rays††								2.6		

* Total abundance assumes that 50% of ^{36}Ar remains trapped within the Earth⁸⁶; the efficiency of degassing for Venus and Mars is scaled by using measurements of ^{40}Ar .

† All mixing ratios are atomic.

‡ Refs 6, 8, 87.

§ Refs 88, 68, 75, for example.

|| Refs 1, 3, and 25. Assumes $^{20}\text{Ne}/^{22}\text{Ne} = 8$ and a mean pressure of 7.5 mbar (ref. 89). The ratio $\text{N}/^{36}\text{Ar}$ in the present atmosphere is approximately 1×10^4 ; the ratio given here refers to the original system and includes estimates for escape of N (see text).

¶ Solar wind data are based on neon observations of Geiss *et al.*⁴⁹, photospheric abundances of neon/argon^{50,90} and lunar data^{53,68} (see text). Solar flare data are from refs 91 and 92.

** Refs 19, 53.

** Refs 14, 20, and 32 with 93 and 94 for neon-E.

†† Refs 95 and 96.

that of the planetary component in meteorites. The low concentration of noble gases in Mars is attributed to differentiation and escape of volatiles from preplanetary martian material.

Our model is based on a postulate that condensation proceeds most rapidly in cooler outer regions of the solar nebula. The solar nebula included from the outset a heterogeneous mixture of gas and particles³⁵⁻³⁹. The quantity of material present in the condensed phase would have increased with time as the nebula cooled. High concentrations of condensate would have appeared first in intermediate regions of the nebula where combinations of low temperature and adequate density favoured most efficient condensation^{40,41}. We suggest that this region occurred near the orbits of Mars and the asteroids. The condensation sequence would have proceeded later near the orbit of Venus as cooling extended to the inner nebula. Coalescence of small particles to kilometre size could have been rapid once the amount of condensed material became comparable with that now in planets^{42,43}. Goldreich and Ward⁴² showed that, if the condensate was confined to a homogeneous disk $<5,000$ km thick, accretion of dust grains into sizeable planetesimals would take place in $\sim 10^3$ yr. As shown below, the noble gas data suggest that conditions necessary for rapid gravitational accretion were present for the pre-Mars material but did not occur in the pre-Venus environment.

The primitive nebula included significant concentrations of ^{26}Al (half life 0.7×10^6 yr)⁴⁴ in addition to trace quantities of short-lived radioisotopes such as ^{107}Pd (ref. 45) and ^{129}I (refs 11, 46). Lee *et al.*⁴⁴ noted that radioactive heat sources in certain meteoritic inclusions were sufficient to melt kilometre sized objects which formed from these materials during the first few million years. Indeed, there is evidence that iron meteorites differentiated within this time period⁴⁵. (Smaller objects characterized by a relatively larger surface area-to-volume ratio could dispose of the excess heat by radiation and would not be expected to attain high internal temperatures.) Objects forming in cooler regions of the nebula would have included high initial concentrations of noble gases^{16,17,47} which would have been

modified subsequently by escape following melting and by input from the solar wind. We suggest that the rapid accretion/melting/escape sequence accounts for the deficiency of noble gas in Mars. In the inner Solar System most of the ^{26}Al would have decayed before formation of kilometre-sized objects. Loss of volatiles from preplanetary Venus material would consequently be negligible.

Venus and the solar wind

Effects of solar wind would have been most important for innermost regions of the nebula. Condensing preplanetary bodies would have incorporated solar wind at a rate proportional to their exposed surface area. According to the present model, the area-to-volume ratio for condensed material would have decreased as a function of distance between the orbits of Venus and Mars. The Poynting-Robertson effect and friction between particles and gas would have led to further concentration of small particles near Venus^{43,48}. We expect therefore that solar wind should have a more important role for Venus than that for Earth and Mars. Our knowledge of noble gases in the solar wind and solar flares is based on a few direct measurements⁴⁹⁻⁵¹ and on detailed analysis of samples from the Moon^{19,52-56}. Lunar soils provide an excellent example of the accumulated effects of irradiation by solar wind and flares, with bulk abundances for ^{36}Ar often as high as 8×10^{-7} g per g. The expected dependence of ^{36}Ar on particle size and exposure time is shown in Fig. 2. Dynamical considerations suggest that the final stages of planetary accretion should occupy a time between 10^7 and 10^8 yr (refs 43,57-59). We could account for ^{36}Ar in Venus' atmosphere from solar wind if Venus formed from materials with a mean radius of 10 m exposed for $\sim 2 \times 10^7$ yr to solar wind of current strength and composition. This material could be distributed uniformly about the orbit of Venus and we would require that it extend ~ 10 planetary radii above the plane of the nebula, if characteristics of the early solar wind were

similar to those of today. The projected surface area and exposure times would be lowered if the early wind were more intense.

One can account therefore in a straightforward fashion for the $^{20}\text{Ne}/^{22}\text{Ne}$ ratio and for the absolute abundance of ^{36}Ar in Venus' atmosphere by postulating a dominant role for the early solar wind. The ratio $^{84}\text{Kr}/^{36}\text{Ar}$ would be $\sim 5 \times 10^{-4}$, consistent with the upper limit reported by Hoffman *et al.*⁶. Indeed it would be difficult to account otherwise for the low value of ^{84}Kr in Venus. The solar wind model has greatest difficulty in accommodating the ratio observed for $^{20}\text{Ne}/^{36}\text{Ar}$. The ratio $^{20}\text{Ne}/^{36}\text{Ar}$

is typically 35 in lunar soils exposed recently to solar wind¹⁹. The abundance is variable for older materials⁶⁰, reflecting loss of neon by diffusion⁶¹. Ratios $^{20}\text{Ne}/^{36}\text{Ar}$ may be as low as 2 and are correlated often with mineral type^{53,55,56}. We shall attempt here to argue that the ratio $^{20}\text{Ne}/^{36}\text{Ar}$ observed by Pioneer Venus, 0.3, may be attributed to differential diffusive loss of neon from pre-Venus material.

The time scale, t , for removal of neon may be represented by

$$t = a^2/D \quad (1)$$

where D is a diffusion coefficient and a is the penetration depth for solar wind, typically $\sim 3 \times 10^{-5}$ cm (ref. 19). Diffusion of noble gas through minerals at temperature T may be characterized by

$$D = D_0 \exp(-E/RT) \quad (2)$$

where R is the gas constant, and E is the activation energy. Values of D_0 for noble gases range from 10^{-3} cm² s⁻¹ in vitreous silica^{62,63} to 10^{-7} cm² s⁻¹ in alumina-glass mixtures⁶⁴. Activation energies^{62,65} vary from 5 to >65 kcal mol⁻¹, and gas may be trapped at individual sites characterized by a spectrum of values for E (refs 66, 67). We expect $^{20}\text{Ne}/^{36}\text{Ar}$ to have an initial value of ~ 35 . The ratio should decrease with time on various time scales t , reflecting applicable values of E_i and the thermal history of the sample.

Assume that Venus formed in $\sim 5 \times 10^7$ yr; neon released from the condensed phase after this epoch would be retained by the planet. Neon evolved earlier would be lost. Assume that the preplanetary material was characterized by a temperature of 333 K (an appropriate temperature for a black body exposed to present sunlight at the orbit of Venus; the actual temperature of preplanetary material could differ depending on conditions in the nebula and the luminosity of the early Sun.) It follows from equations (1) and (2) that neon trapped at sites with $E_i < 26$ kcal mol⁻¹ would be lost; neon at sites with $E_i > 26$ kcal mol⁻¹ would be retained. Studies based on step heating of lunar samples⁶⁸⁻⁷⁰ suggest that $>95\%$ of neon implanted in preplanetary Venusian material would be lost in 2×10^7 yr at 333 K (equivalent to 1 h at 1,050 K). Loss of ^{36}Ar and ^{84}Kr would be negligible, see Fig. 3. The resulting value for $^{20}\text{Ne}/^{36}\text{Ar}$ should lie between 0.5 and 2.0, consistent with the observed value⁶, 0.3 ± 0.2 .

The neon problem

The ratio $^{20}\text{Ne}/^{22}\text{Ne}$ is variable in the Solar System, with values typically between 7 and 14 (see Table 1). The range of values observed for $^{20}\text{Ne}/^{22}\text{Ne}$ is thought to reflect fractionation of neon in the primitive nebula¹² and, in addition, heterogeneity associated most probably with material synthesized in different nuclear environments⁷¹⁻⁷⁴. (Note that the ratio $^{36}\text{Ar}/^{38}\text{Ar}$ is remarkably uniform for all materials sampled to date^{6,14,37}; it is unlikely therefore that the variety in $^{20}\text{Ne}/^{22}\text{Ne}$ can be attributed solely to diffusive separation¹².) There is uncertainty concerning the value for the $^{20}\text{Ne}/^{22}\text{Ne}$ ratio of the Sun and presumably for the original nebula. Solar flares show a ratio of ~ 8 , while the ratio in solar wind is 13.6. It is unclear which, if either, of these values is representative of the Sun. A ratio $^{20}\text{Ne}/^{22}\text{Ne}$ of ~ 8 is typical for the planetary component in meteorites and seems to be favoured for condensation in the early nebula. Materials modified by solar wind exhibit ratios in the range 11–14. We could account for the terrestrial ratio of 9.8 if 40% of Earth's neon were derived from solar wind, with the remainder drawn from the planetary component. We might expect preplanetary Earth to retain solar wind neon with higher efficiency than Venus, as temperatures and effective diffusion coefficients would have been lower at the orbit of Earth. Sources of ^{36}Ar , ^{84}Kr and ^{132}Xe from the solar wind would be negligible compared with quantities of these gases trapped during condensation. We could account for terrestrial neon if preplanetary material of mean radius 50 km were exposed to solar wind for 2×10^7 yr.

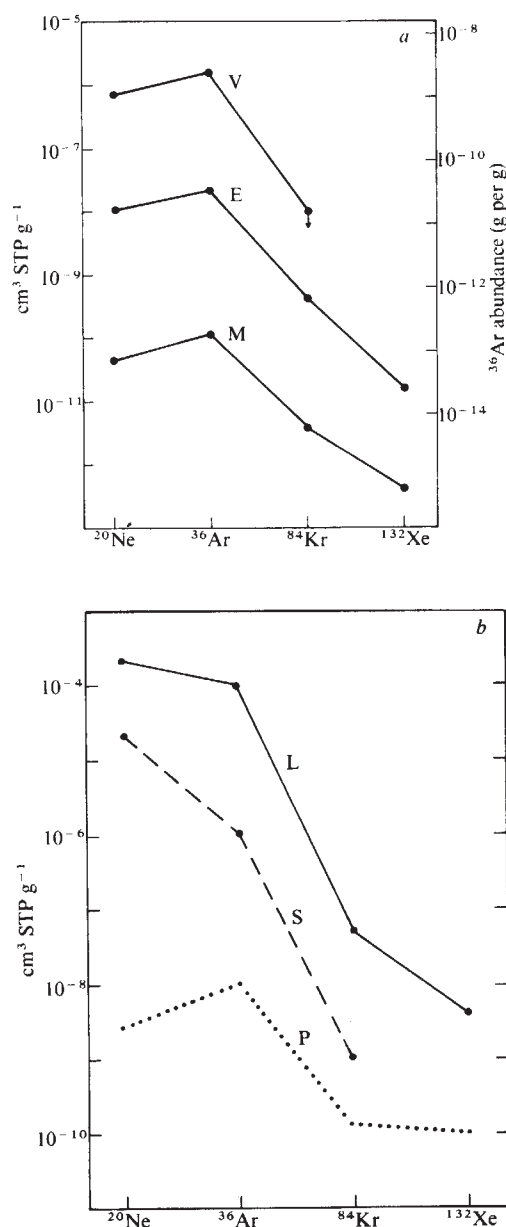


Fig. 1 *a*, Abundances of primordial noble gases observed in the atmospheres of Venus (V), Earth (E) and Mars (M). Abundances are quoted as ratios referenced to mass of the parent planet. The abundance of ^{20}Ne for Mars is based on an assumed ratio $^{20}\text{Ne}/^{22}\text{Ne}$ of 8. The abundance indicated for ^{84}Kr on Venus is an upper limit. *b*, Relative abundances of primordial noble gas for lunar fines (L), the solar component (S) found in 'gas-rich' meteorites and the planetary component of meteorites (P). Abundances of ^{36}Ar for L, S and D are set arbitrarily equal to 10^{-4} , 10^{-6} and 10^{-8} , respectively. For absolute abundances of ^{36}Ar see Table 1.

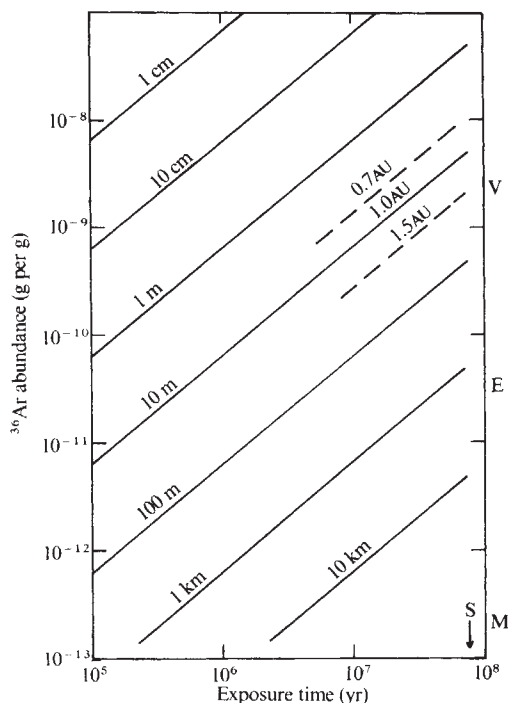


Fig. 2 Mean abundance of ^{36}Ar (g per g) accumulated by planetesimals from the solar wind as a function of exposure time. Planetesimals are assumed to follow an exponential distribution in radius ($N(r)dr = \exp(-r/a)dr$) and to have a mean density of 3 g cm^{-3} . Each solid line refers to a particular distribution with mean radius a as given. Abundances are quoted for exposure to present solar wind at the orbit of Earth (1.0 AU). The effect of location is shown for a mean radius of 10 m, with data for Venus (0.7 AU) and Mars (1.5 AU) indicated by dashed lines. The saturation limit⁸⁵ $10^{-2}\text{ cm}^3\text{ STP of gas cm}^{-2}$, is denoted by S. Abundances of ^{36}Ar for the atmospheres of Venus (V), Earth (E) and Mars (M) are indicated.

Mars

We expect neon implanted by the solar wind to be retained with relative ease by preplanetary Mars. The solar wind component would be characterized by a ratio $^{22}\text{Ne}/^{36}\text{Ar}$ of about 1 (refs 19, 54, 68, 75) in contrast to the value 0.047 observed for Mars³. We might hope to use these data to place limits on the time for effective exposure of preplanetary material to solar wind at the orbit of Mars. The data in Fig. 2 and Table 1 suggest that 100-km objects could be exposed to solar wind for no more than $5 \times 10^5\text{ yr}$, a somewhat paradoxical result at first sight. We require preplanetary Mars material to melt and differentiate early, and its average size to be small enough to permit efficient escape of gases as heavy as xenon. On the other hand, dynamical considerations require that final stages of planetary accretion should occupy a time interval of at least 10^7 yr (refs 43, 57, 59). It is likely that planetesimals at Mars' orbit may have been shielded to some extent from solar wind by material in the inner Solar System. The paradox may also be resolved if ionization of gases evolved from preplanetary bodies were to provide a shield insulating surfaces from solar wind^{76,77}. Indeed this shield must be operative at present as the flux of ^{22}Ne in the current solar wind would account for the abundance of neon in Mars' atmosphere in as little as 10^7 yr .

The ratio $^{129}\text{Xe}/^{132}\text{Xe}$ is 2.5 for Mars, 0.98 for Earth, 1.05 for solar wind, and ranges between 0.6 and 3.0 for bulk meteorites with much higher values for specific inclusions^{3,11,12,68,75,78-80}. High values of $^{129}\text{Xe}/^{132}\text{Xe}$ require a mechanism to deplete xenon relative to iodine in materials forming Mars and certain meteorites. Depletion must occur within $3 \times 10^7\text{ yr}$ following incorporation of ^{129}I in planetesimals. The relatively high value

for $^{40}\text{Ar}/^{36}\text{Ar}$ in Mars' atmosphere seems to require a corresponding mechanism to deplete argon relative to potassium and is explained by the melting/degassing scenario envisaged here.

Mars' atmosphere is composed mainly of CO_2 with trace quantities of N_2 , H_2O and photochemical products such as CO , O_2 and O_3 , in addition to the noble gases discussed above^{2,3,81,82}. Nitrogen is especially interesting. Its isotopic composition differs significantly from that of Earth and other Solar System materials. The difference is attributed to preferential escape of ^{14}N relative to ^{15}N , and implies an initial abundance for atmospheric N_2 of no less than $8 \times 10^{22}\text{ molecules cm}^{-2}$, more probably $\sim 1.3 \times 10^{24}\text{ molecules cm}^{-2}$ (refs 1, 83). One might assume that nitrogen is released from the solid body with an efficiency only slightly less than that for argon. In this case nitrogen in the early Mars would be $\sim 2.6 \times 10^{-6}\text{ g per g}$, certainly no less than $1.6 \times 10^{-7}\text{ g per g}$, with 5% of the total in the primitive atmosphere. The ratio $\text{N}/^{36}\text{Ar}$ would be at least as large as 1×10^5 , more probably $\sim 2 \times 10^6$. By way of comparison the ratio is 5×10^4 for Earth and 2.5×10^3 for Venus (see Table 1). The low value for Venus is attributed to addition of argon from the solar wind in the preplanetary phase, a process which would not significantly affect nitrogen. The high value for Mars could

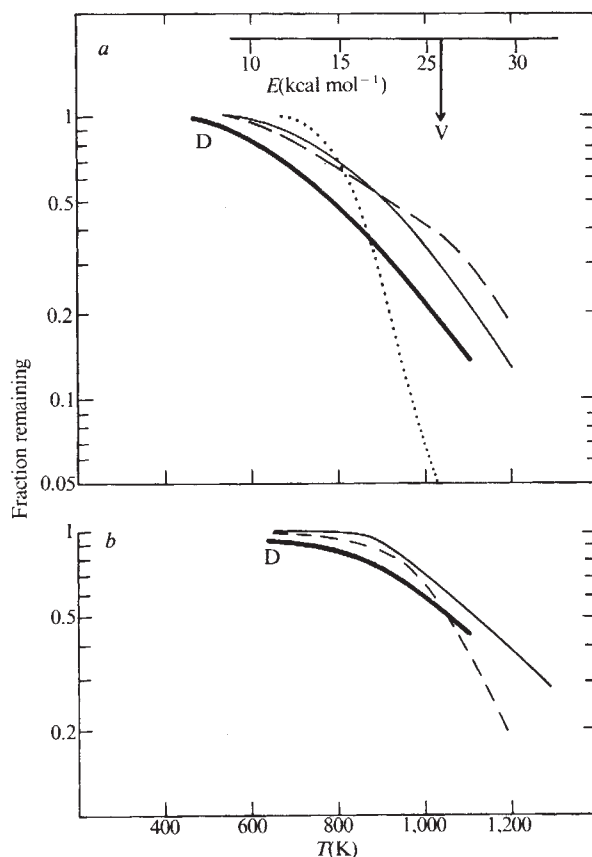


Fig. 3 Fraction of noble gas remaining in lunar samples after heating at a given temperature for 1 h. The initial $^{20}\text{Ne}/^{36}\text{Ar}$ ratio in these materials is typically 6. The mean rate of degassing of neon and krypton was measured for three lunar samples by Drozd *et al.*⁷⁰ and is indicated by the thick line D (^{20}Ne in a, ^{84}Kr in b). Other data are from Pepin *et al.* (ref. 69). Solid lines are for lunar samples 10084 (^{22}Ne in a, ^{84}Kr in b); dashed lines, lunar rock 10069 (^{22}Ne in a, ^{36}Ar in b); dotted line, lunar breccia 10061 (^{22}Ne in a). The energy scale refers to the activation energy of an implanted gas which would be released in the following conditions: heated at the given temperature for 1 h following implantation at a mean depth of $3 \times 10^{-5}\text{ cm}$, with $D_0 = 10^{-7}\text{ cm}^2\text{ s}^{-1}$ (see text). The arrow labelled V denotes the maximum activation energy consistent with relatively complete escape of gas in $5 \times 10^7\text{ yr}$ from an object at the orbit of Venus (333 K), see text.

reflect preferential retention of chemically bound N (ref. 84) relative to Ar during melting.

Conclusions

We may summarize now our response to the questions raised earlier: (1) the abundance of neon and argon in Venus' atmosphere is dominated by implantation of solar wind in preplanetary material; (2) the deficiency of primordial noble gases in Mars' atmosphere is attributed to differentiation of preplanetary material; (3) the origin of Venus' neon is different from that of Earth and Mars and the similarity of Ne/Ar for Venus to values for the other inner planets is fortuitous; (4) the discrepancy between Venus' noble gases and the planetary pattern is due to large additions of gas from preplanetary solar wind; (5) the ratio $^{40}\text{Ar}/^{36}\text{Ar}$ for Mars is higher than for Earth due to escape of ^{36}Ar in the preplanetary phase, and the higher value for $^{129}\text{Xe}/^{132}\text{Xe}$ arises because Mars formed early with high concentrations of ^{129}I ; (6) Venus' neon is derived from solar wind and reflects its isotopic composition; (7) the solar wind would not add significant quantities of nitrogen and the high value of $^{36}\text{Ar}/\text{N}$ on Venus is attributed to excess ^{36}Ar . Our model suggests that the pattern of noble gases for Mars should resemble the planetary pattern of meteorites, that the ratio $^{20}\text{Ne}/^{22}\text{Ne}$ should be ~ 8 . Krypton and xenon on Venus would be derived in part from solar wind, in part by condensation from the primitive nebula. We expect a ratio $^{132}\text{Xe}/^{84}\text{Kr}$ in Venus'

atmosphere of ~ 0.1 , intermediate between the values observed for Earth and solar wind, with $^{129}\text{Xe}/^{132}\text{Xe} \sim 1$.

More precise observations are required to test and develop these ideas. It is clear that they could be refined and would be more quantitative if formulated in the context of a comprehensive physical model of planetary formation. There are reasons to expect, in turn, that observations of noble gases in meteorites, comets, asteroids and planets should provide invaluable clues to processes in the early nebula, aiding significantly in the development of such a model.

The ideas in this paper were presented in various forms at the 1980 Solvay Conference in Chemistry (April 1980), the 23rd Annual COSPAR Meeting (June 1980) and the Sun and Climate Conference (October 1980). We thank many colleagues, notably E. Anders, T. M. Donahue, M. Krook, T. Owen and G. J. Wasserburg, for criticism. M.B.M. thanks particularly A. O. Nier and J. H. Hoffman, principal investigators on the mass spectrometric experiments conducted by Viking and Pioneer Venus. We thank F. Forrestel Zingale for help in the preparation of the manuscript. This research was supported by NSF grant ATM79-13251.

Note added in proof: After completion of this work we learned of an independent conclusion by G. Wetherill⁹⁷ that Venus' ^{36}Ar is derived from solar wind. Further analyses of the Pioneer data by Donahue *et al.*⁹⁸ give ratios for $^{84}\text{Kr}/^{36}\text{Ar}$, $^{132}\text{Xe}/^{84}\text{Kr}$ and $^{129}\text{Xe}/^{132}\text{Xe}$, of 10^{-3} , 0.25, and about 1, respectively, in excellent agreement with the model discussed here.

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Microinjection of cloned retroviral genomes into mouse zygotes: integration and expression in the animal

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Cloned genomic retroviral DNA is expressed in mouse zygotes after microinjection. The resulting virus integrated into the mouse genome at a single chromosomal site and was expressed in a tissue-specific manner. Our findings suggest that the chromosomal position of a viral genome influences its activation during development.

IN recent experiments in our laboratory, we have used the techniques of microinjection of Moloney leukaemia virus (M-MuLV) into preimplantation mouse embryos (4–30-cell stage) as a model for the insertion of DNA into the germ line of mice^{1–5}. One serious complication in that approach is the extensive mosaicism of the animals which result, so that genetically defined substrains can be obtained only after segregation in the next generation. From one of these strains, the Mov-3 substrain, which carries the MuLV at the *Mov-3* locus⁴, we have isolated a 16.8-kilobase (kb), *EcoRI* fragment containing viral and flanking mouse sequences⁶ which is highly infectious in a mouse fibroblast transfection assay. We now describe how cloned Mov-3 DNA microinjected into one-cell mouse embryos can lead to the production of animals carrying a single inserted genome copy in each cell and which is expressed in a tissue-specific manner.

Microinjection of DNA and expression in the animal

Mouse zygotes of strain C57BL/6 were isolated 12–16 h after fertilization and $\sim 2\text{--}4 \times 10^{-6}$ μl of Mov-3 DNA (600 $\mu\text{g ml}^{-1}$) dissolved in 100% glycerol⁷ were microinjected into the cytoplasm. The DNA was prepared by digesting pMov-3 with *EcoRI* to cleave the M-MuLV-specific sequences flanked by mouse-specific sequences from the pBR322 vector. After microinjection the embryos were transferred to the oviduct of day-1 pseudopregnant females.

Five females became pregnant after having received a total of 75 embryos and gave birth to 30 mice. At 4 weeks of age the animals were tested for viraemia (infectious virus in the blood) by radioimmunoassay³. Viraemia is a sensitive assay for virus activation, as a single infectious virus particle is sufficient to initiate a cycle of virus replication and subsequent virus spread through all susceptible cell populations. Three of the mice were viraemic, one of which (no. 158) was killed when 5½ months old, and DNA and RNA were extracted from different tissues. Autopsy revealed that the animal had not yet developed leukaemia (spleen weight 0.10 g; thymus 0.03 g). Viral-specific RNA was expressed in all the organs of the animal which were analysed⁸, but at different concentrations (Fig. 1 and Table 1). The amounts of virus-specific RNA were highest in muscle, spleen, lung and testes. In Mov-1 mice, expression occurs predominantly in lymphatic tissues⁸ and in Mov-3 mice in lymphatic and non-lymphatic organs (Table 1).

Structure of M-MuLV DNA

There are at least three different pathways that could lead to expression of microinjected DNA: (1) the microinjected DNA

could integrate into the mouse genome at an early stage of development; (2) the DNA could persist during part of development and be expressed later, leading to synthesis of infectious virus which would spread to all susceptible cells, resulting in virus expression in many different organs (see refs 5, 9); (3) the Mov-3 DNA could be expressed after infection, and infectious virus integrate into the genome of the zygote or two-cell embryo.

To distinguish between these possibilities, DNA from different tissues was digested with a restriction enzyme having no recognition site in M-MuLV proviral DNA (*EcoRI*)¹⁰. If the injected DNA or virus has integrated at the zygote or two-cell stage (possibilities 1 and 3), M-MuLV-specific *EcoRI* fragments should be present in molar amounts in all tissues. Possibilities (1) and (3) are distinguishable by mapping the integration site with different enzymes. If virus has integrated at a later stage of embryogenesis [possibility (2)], virus integration would have taken place at different chromosomal sites in individual cells. This would result in submolar concentrations of individual M-MuLV fragments¹¹, and so no specific bands should be detected after *EcoRI* digestion.

Figure 2A shows that all organs of mouse 158 contained a single M-MuLV-specific 24-kb *EcoRI* fragment that hybridized

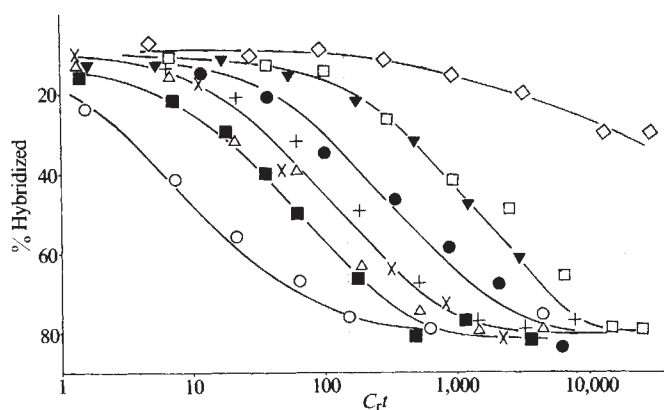


Fig. 1 Annealing kinetics of cellular RNA from different organs of mouse 158 (Δ , spleen, thymus; +, testis; $\times$, lung; $\circ$, muscle; $\blacktriangledown$, brain; $\square$, liver; $\bullet$, kidney). As a control, RNAs from spleen of BALB/Mo ($\blacksquare$, Mov-1) and BALB/c ($\diamond$) mice were included in the analysis. Isolation of RNA and annealing conditions to representative M-MuLV cDNA have been described previously⁸. The results are expressed as percentage hybridized (as measured by resistance to S_1 digestion) at different C_t values which were then corrected to standard annealing conditions.

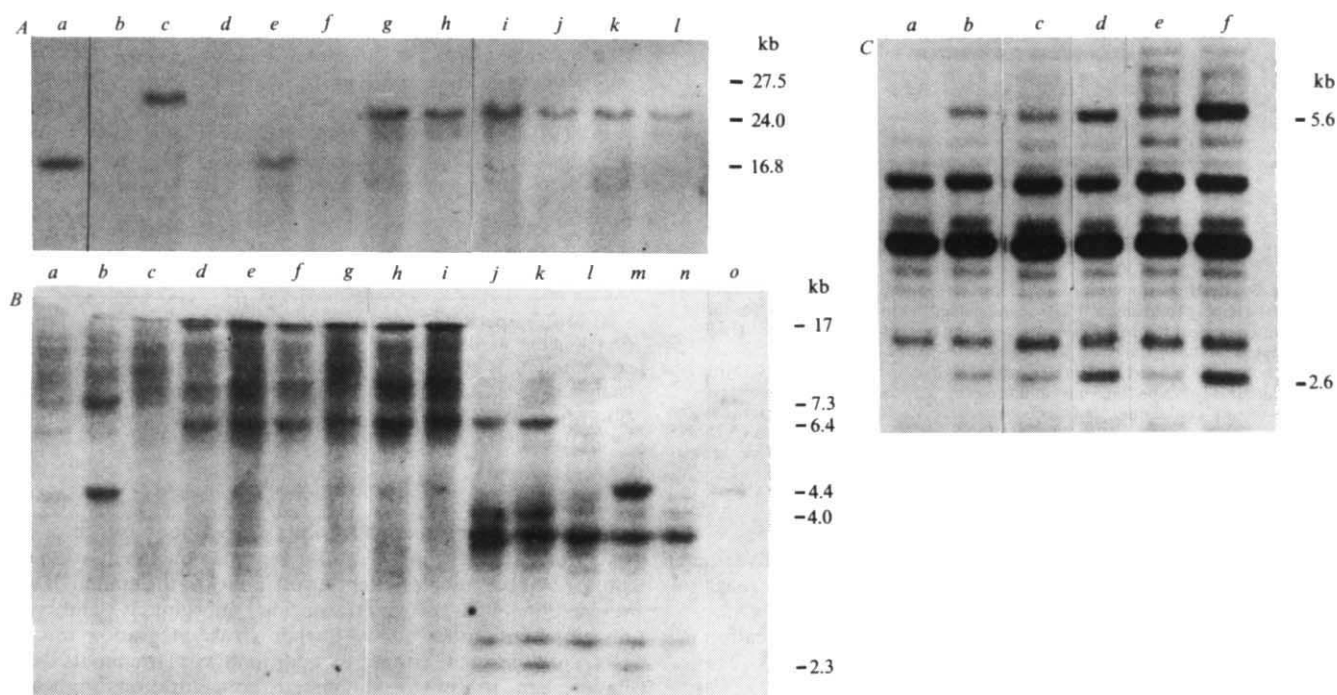


Fig. 2 Restriction enzyme analysis of DNA from mouse 158. **A**, digestion with restriction enzyme *EcoRI*. High molecular weight DNA was prepared as described previously¹¹ and 15- μ g samples were cleaved with an excess of endonuclease *EcoRI* (Boehringer) and then run on a 0.5% agarose gel. DNA fragments were transferred to a nitrocellulose filter by the method of Southern²¹ and hybridized to M-MuLV-specific cDNA¹¹. Lanes *a*, 200 pg of *EcoRI*-cleaved pMov-3; *b*, BALB/c liver DNA; *c*, Mov-1 liver DNA; *d*, ICR liver DNA; *e*, Mov-3 liver DNA; *f*, C57BL liver DNA; *g-l*, DNAs from muscle, testis, brain, liver, spleen kidney respectively of animal 158. The length of the M-MuLV-specific *EcoRI* fragments of Mov-1 and Mov-3 animals has been determined previously and these fragments were used as size markers. **B**, digestion with restriction enzymes *EcoRI*, *HindIII* and *BclI*. Experimental conditions were as described for **A**, except that a 0.8% agarose gel was used. All DNAs were digested with an excess of *EcoRI* and *HindIII*. After phenol treatment and ethanol precipitation some DNAs were further digested with an excess of *BclI* (Bethesda) as shown in lanes *j-o*. Lanes *a*, ICR DNA; *b*, Mov-3 DNA; *c*, C57BL DNA; *d-i*, DNAs from kidney, spleen, liver, brain, testis and muscle respectively of animal 158; *j*, *k*, DNAs from spleen and brain respectively of animal 158; *l*, C57BL DNA; *m*, Mov-3 DNA; *n*, ICR DNA; *o*, 200 pg pMov-3. The M-MuLV-specific *HindIII* fragments of Mov-3 (7.3 kb and 4.4 kb; see Fig. 3) were used as size markers. *BclI* cleaves once in the M-MuLV genome (see map in Fig. 3); however it does not cleave viral DNAs such as pMov-3, which have been cloned and propagated in *Escherichia coli*, because the adenine residues in its recognition sequence are methylated²². Lane *o* shows that the *HindIII* fragments of the cloned Mov-3 DNA are not cleaved by *BclI*, in contrast to the 7.3-kb *HindIII* fragment of DNA from Mov-3 mice (lane *m*). Lanes *j* and *k* show that the 17-kb *HindIII* fragment present in the DNA of mouse 158 is also cleaved by *BclI*, resulting in two new M-MuLV-specific fragments of 4 and 2.3 kb. The 2.3-kb fragment represents the *HindIII*-*BclI* fragment present in M-MuLV proviral DNA (see map in Fig. 3). **C**, digestion with restriction enzyme *SacI*. Experimental conditions were as described for **A**, except that a 0.8% agarose gel was used and the filter was hybridized with a nick-translated cloned M-MuLV DNA. Lanes *a*, C57BL liver DNA; *b-d*, DNAs from muscle, liver and spleen respectively of animal 158; *e*, Mov-3 liver DNA; *f*, DNA from spleen of a preleukaemic Mov-3 animal. The Mov-3 mice were heterozygous for the Mov-3 locus.

with equal intensity to M-MuLV-specific cDNA. The fragment differed in size from both Mov-3 DNA (16.8 kb, Fig. 2A, lane *a*) and Mov-1 DNA (27.5 kb, Fig. 2A, lane *c*), suggesting a different integration site. This was confirmed by analysing the flanking sequences in digestion experiments with *EcoRI* and *HindIII* (Fig. 2B, compare lanes *a-i*) or *EcoRI*, *HindIII* and *BclI* (lanes *j-o*). Other restriction enzymes (not shown in Fig. 2B) were used to construct a restriction map of the integrated proviral DNA (Fig. 3). Experiments using labelled pBR322 DNA as a probe failed to show the presence of vector sequences in the animal DNA (data not shown).

The restriction enzyme analysis indicated that Mov-3 and vector DNA did not integrate into the mouse genome, but was lost during subsequent development, suggesting that the cloned DNA was transcribed after microinjection and that virus integrated *de novo* into the mouse genome at an early stage of development, probably at the zygote stage. The lack of mosaicism is in sharp contrast to animals derived from embryos exposed to M-MuLV at the 4–30-cell stage, in which submolar concentrations of M-MuLV were observed in somatic tissues⁴ as well as in the germ line^{3–5}.

Two mechanisms could explain the different levels of virus-specific genome expression in the tissues of mouse 158. The 24-kb *EcoRI* fragment carrying the M-MuLV genome could be expressed at a high level in some tissues and at a low level or not at all in others (see Table 1). Alternatively, infectious virus could

have been activated at some time during embryogenesis leading to superinfection of all susceptible cells and subsequent expression of the reintegrated viral copies. The latter mode of virus expression has been demonstrated for Mov substrains of mice which carry somatically acquired M-MuLV copies in their lymphatic tissues (Mov-1, Mov-3, Mov-9, Mov-13) and in some non-lymphatic tissues (Mov-3, Mov-9, Mov-13)^{5,8,9}. Recent evidence has suggested that virus expression in the different tissues of these mouse substrains was due to the presence of the somatically acquired M-MuLV copies and not the endogenous M-MuLV copy⁹.

To decide whether the tissue-specific expression of M-MuLV-specific RNA resulted from the presence of multiple M-MuLV copies, possibly acquired by superinfection, the total number of M-MuLV copies present in the DNA of each tissue was estimated. As M-MuLV copies acquired by superinfection would integrate at many different sites in individual cells, *EcoRI* digestion would produce submolar concentrations of individual M-MuLV fragments¹¹, so that no specific bands would be detected. Digestion of the DNA with an enzyme that cuts within the M-MuLV genome, *SacI*, yields two internal M-MuLV-specific fragments of 5.6 and 2.6 kb⁵. These fragments combine all integrated M-MuLV sequences regardless of where integration occurs. The intensity of these fragments is then an estimate of the total number of M-MuLV copies present in DNA of each tissue, with the *SacI* fragments of endogenous murine C-type

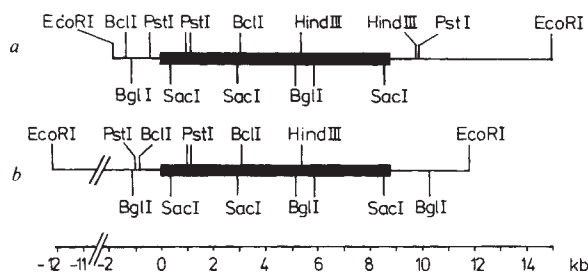


Fig. 3 Restriction enzyme map of the integrated M-MuLV provirus of Mov-3 mice (a) and mouse 158 (b). The shaded bar spanning 0–8.9 kb on the map represents the M-MuLV provirus. The map of the integrated Mov-3 provirus has been published previously⁶. The map of the integrated provirus of mouse 158 was derived by cleavage of high molecular weight DNA with different restriction enzymes followed by agarose gel electrophoresis. Fragments containing viral sequences were identified by blot transfer hybridization with a ³²P-labelled cDNA that was specific for M-MuLV¹¹. Only the restriction sites within and directly adjacent to the provirus are shown.

Table 1 Relative concentration of M-MuLV-specific RNA in organs of mouse 158 and in Mov-1 and Mov-3 mice

Organ tested	Mouse 158	Mov-1	Mov-3
Spleen	300	250	300
Kidney	70	1	10
Liver	15	1	4
Brain	16	0.5	5
Muscle	1,500	0.5	25
Testis	125	NT	10
Lung	250	5	50

M-MuLV-specific RNA sequences were quantified from reassociation kinetics as described in Fig. 1 legend. M-MuLV-specific RNA concentration is given as a relative concentration to the RNA concentration in livers of newborn Mov-1 mice, which was set as 1 (corresponding to a 1/2 C_t of 15,000)⁸. The values for Mov-1 mice are taken from previous publications⁸. NT, not tested.

viruses acting as an internal standard. Figure 2C (lanes b–d) shows that the M-MuLV-specific *SacI* fragments in liver and muscle DNA of mouse 158 were of equal intensity, whereas the spleen fragments were stronger. As a control, DNAs of a Mov-3 animal were included showing M-MuLV DNA amplification in spleen when compared with liver (Fig. 2C, lanes e and f). These observations confirm that viral RNA expression on lymphatic tissues of viraemic animals is accompanied by somatic DNA amplification^{8,9,11} and could be due to transcription of the M-MuLV genome in the 24-kb fragment and/or of M-MuLV copies acquired by superinfection. No M-MuLV DNA amplification, however, was seen in muscle of mouse 158, which expressed 100 or 5 times more viral RNA compared with liver or spleen respectively (Table 1). Our results suggest that viral RNA expression in muscle was due to specific expression of the M-MuLV genome in the 24-kb *EcoRI* fragment and not to superinfection of muscle cells with virus. We cannot exclude, however, that superinfection of a small fraction of cells took place which was not detected by the present assay. If this was the case, the acquired M-MuLV copies would have to be expressed at much higher rates or would have to synthesize more stable viral RNA than M-MuLV copies of lymphatic tissues.

Conclusions

Early mouse embryos, as well as undifferentiated teratocarcinoma cells, seem to be non-permissive for virus replication after infection with DNA^{12–15} or RNA^{16–18} tumour viruses. Microinjection of SV40 DNA into blastocysts did not lead to virus expression in the resulting animals¹². However, expression of cloned *Xenopus* DNA microinjected into unfertilized mouse oocytes has been reported¹⁹. Our experiments provide the first demonstration that cloned DNA can be expressed when injected into a zygote leading to synthesis of infectious virus and virus integration. The injected DNA did not integrate and was lost during subsequent development, as we did not detect either Mov-3 flanking mouse sequences or vector sequences. In support of earlier conclusions¹, the integrated virus genome was probably not expressed, and no virus spread occurred, during

early embryogenesis. This is indicated by the presence of only one viral copy per diploid mouse genome equivalent at a single site (=24-kb *EcoRI* fragment) in all tissues of the animal. If virus replication had occurred during subsequent embryonal development, followed by further virus integration into the DNA of different blastomeres, additional viral fragments should have been present in submolar concentrations indicating somatic mosaicism. Mosaicism has been demonstrated invariably in animals derived from M-MuLV-infected 4–30-cell embryos^{3–5}. However, the integrated genome was expressed during later developments in a tissue-specific manner, which was different from M-MuLV expression in Mov-3 mice (Table 1). This supports the hypothesis that the chromosomal position at which a viral genome is integrated influences its expression during development and differentiation^{4,5}.

It was recently claimed that recombinant DNA microinjected into mouse zygotes persisted in later embryos. The data, however, were difficult to interpret with respect to the structure and origin of the recombinant DNA found in the newborns²⁰. We find a general problem in such experiments—the possibility of plasmid contamination. The use of the enzyme *BclI*, which is sensitive to methylation in adenine, indicated clearly that the M-MuLV-specific sequences observed in mouse 158 originated from chromosomal sequences and did not result from laboratory contamination with DNA of prokaryotic origin (Fig. 2B).

The most efficient way to insert new genetic information into the germ line seems to be introduction of genes into early embryos^{3–5}. An unresolved problem of this approach for 'gene therapy' is in predicting the expression of the inserted gene in a given tissue, and this may only be possible by understanding the molecular mechanisms that determine expression and integration of DNA in the injected embryo.

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Nucleotide sequence of Moloney murine leukaemia virus

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We have determined the 8,332-nucleotide structure of the genome of Moloney murine leukaemia virus. The coding frame for the gag gene is the same as that for pol, separated only by a single amber triplet. The pol gene encodes 132,000 daltons of protein, larger than the 70,000 daltons required for the reverse transcriptase. The env gene overlaps the pol gene.

RETROVIRUSES have long been implicated in the aetiology of various types of neoplasia. Moloney murine leukaemia virus (Mo-MuLV) is a prototype mammalian type C virus originally isolated from a sarcoma passaged in BALB/c mice¹. Injection of Mo-MuLV into newborn mice results in virtually all the mice developing a generalized lymphocytic leukaemia within 3 months¹. Mo-MuLV differs from the more rapidly acting, defective, leukaemogenic and sarcomagenic retroviruses in that it possesses a full complement of genes and is capable of autonomous replication. Essential to the understanding of the mechanism(s) by which Mo-MuLV can induce leukaemia is the elucidation of its genetic structure, gene products and gene functions.

After infection, the viral RNA genome is reverse transcribed to produce a double-stranded DNA copy which can integrate into the host chromosome to form the provirus. In outline, the structure of the Mo-MuLV provirus is 5'-long terminal repeat (LTR)-gag-pol-env-LTR-3' (Fig. 1; see refs 2, 3, for review). The provirus is transcribed to produce the plus-strand, genomic RNA⁴. The genes, gene order and gene products have been determined by a combination of biochemical, genetic and immunological methods (for review see ref. 5). The precise structure of the Mo-MuLV LTRs has been deduced from the nucleotide sequences of the ends of Mo-MuLV cDNA⁶, Mo-MuLV provirus⁷ and provirus of the related Moloney murine sarcoma virus⁸. We now report the nucleotide sequence of a full-length clone of Mo-MuLV, the analysis of which allows us to define the complete genetic structure of Mo-MuLV.

General features of the sequence

Figure 2 presents the DNA sequence of plasmid pMLV-1 that corresponds to the viral genome—that is, the plus or sense strand of viral RNA. The base designated number 1 represents the 5' end of the genome and has been identified as the methylated base to which the 5' cap (7meGppp) is added⁹. The 3' end of the viral RNA is polyadenylated¹⁰. The precise nucleotide to which the poly(A) tail is added is unknown, but we assign the dinucleotide CA at positions 8,331–8,332 as the poly(A) addition site because: (1) the dinucleotide CA is the preferred poly(A) addition site¹¹, (2) this dinucleotide is 15 residues from the poly(A) addition signal (AATAAA) at positions 8,311–8,316, the preferred distance¹¹, and (3) the dinucleotide is 3' to the RNase T₁ oligonucleotide mapped as the most 3'-proximal oligonucleotide¹². These assignments define the virus-encoded genome as having 8,332 nucleotides. The 5' cap and 3' poly(A) tail are in addition to the 8,332 nucleotides.

The ends of the viral genome have key roles in virus replication and integration^{2,3}. As their structures have been

described in detail elsewhere^{13,14}, we will simply point out these structural features in the context of their biological roles. After infection, reverse transcription of the Mo-MuLV genome shown in Fig. 2 would produce an 8,858-base pair long DNA copy of the genome in which the 594 bases at either end are identical. These long terminal repeats (LTRs) are the by-product of the mechanism of reverse transcription. Their precise structure results from the relative positions of the origins of replication to the terminally redundant RNA sequence that is required for the 'strong-stop' DNA molecule to be able to jump to the 3' end of the template and complete DNA synthesis. The LTR structure is represented by convention as U₃-R-U₅ (refs 2, 3). The U₃ region contains bases uniquely present at the 3' end of the viral genome (7,816–8,264) and corresponds to those bases between the origin of (+)strand DNA synthesis and the terminally redundant RNA sequence. The R region sequence appears once at the 3' end (8,265–8,332) and once at the 5' end (1–68) of the viral genome and supplies the redundancy necessary for the jump during reverse transcription^{12,15–18}. The U₅ region contains bases uniquely found at the 5' end of the genome (69–145) and corresponds to those nucleotides between the origin of (–)strand DNA synthesis (that is, tRNA^{Pro} primer binding site 146–163) and the repeated sequence (R).

The double-stranded DNA copy of the genome (LTR-gag-pol-env-LTR) is transported to the nucleus where at least part of it is converted to closed circular molecules¹⁹. It is not known whether it is the circular or linear form that can integrate into the host chromosome to generate a provirus^{20,21}. The integrated proviral structure is nearly identical to that of the linear molecule (cell DNA-LTR-gag-pol-env-LTR-cell DNA) except it has lost two bases from each end of the linear molecule^{8,14,22,23}.

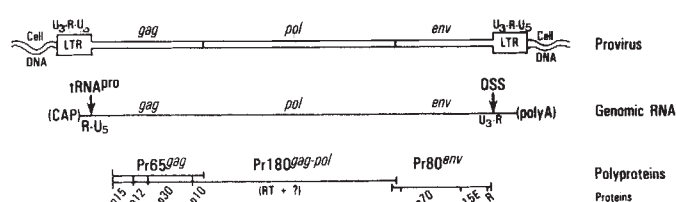


Fig. 1 Structure of Mo-MuLV provirus and genomic RNA. The origins of plus (oss) and minus (tRNA^{Pro}) strand DNA synthesis are indicated on the genomic RNA, as well as the structural features of LTR. Pr65^{gag} and Pr180^{gag-pol} are translated from a 35S mRNA and Pr80^{env} from a spliced, 21S mRNA. The mature protein species of the three primary polyproteins are indicated (RT is reverse transcriptase, the question mark indicates that other pol products are likely but as of yet uncharacterized). The env gene overlaps the 3' end of the pol gene.

Hence, the provirus is 8,854 base pairs (bp) long. As the linear molecule and the LTRs have structures analogous to those of transposable genetic elements^{6-8,14,22,23} and as integration produces a 4-6-bp duplication of the host sequences at the insertion site^{8,14,22,23}, integration is thought to occur by a mechanism similar to that used by transposable genetic elements (see ref. 24 for review). Hence, by analogy, we assume that the 13-base long inverted repeats which flank the LTRs (bases 7,816-7,828 in the U₃ region and 133-145 in the U₅ region) have a key structural role in the integration process.

Transcription of the provirus, which is initiated in the 5'LTR and terminated in the 3'LTR, generates the viral mRNAs and the genomic RNA. As such, the LTRs contain the signals necessary for transcription of the provirus. Three sequences characteristic of eukaryotic transcription initiation signals are found at approximately -70, -25 and +8 bases relative to the 5' end of the mRNA²⁵. A match to each paradigm sequence is present in the appropriate place in the LTR: the sequence CCAAT at -81 to -77 (in the U₃ region, thus at 8,184-8,188 in the genome) is a 5/5 match with the paradigm CCAAT; the sequence AATAAAAG at -30 to -23 (U₃, 8,235-8,242 in the genome) is a 5/8 match with the Hogness-Goldberg box, TATAAATA; and the sequence CCTCCG at +9 to +14 (R region) is a 5/6 match with the consensus sequence CTTYG. The 3' end of the mRNA molecule was assigned such that the poly(A) addition signal sequence (AATAAA) was 17-22 nucleotides upstream. As this sequence is in the R region of the LTR, it occurs both at positions 8,311-8,316 at the 3' end and at 47-52 at the 5' end of the genome. As expected for such a large structure, these several consensus sequences are also present in other regions of the genome. We have not assigned any biological role to these scattered matches to the consensus sequences.

The LTR in this clone of Mo-MuLV contains a 75-base tandem duplication; that is, in the U₃ region bases 7,933-8,007 are repeated at positions 8,008-8,082. A similar duplication of 73 bp is seen in the U₃ region of the LTR of Moloney sarcoma virus⁸, but only one copy of this duplicated sequence is seen in the LTRs of a clone of integrated Mo-MuLV⁷ and a cDNA clone of Mo-MuLV⁶. The single copy in the latter two clones is flanked by 10-base direct repeats. The tandem duplication found in the present sequence has three copies of this direct repeat at 7,923-7,932, 7,998-8,007 and 8,073-8,082. The positions of the direct repeats with respect to the single copy and tandemly duplicated sequences suggest that the tandem duplication arose by an unequal crossing-over event involving two LTRs with single copies of the sequence. If such a mechanism is involved, this portion of the U₃ region may be a rapidly changing part of the LTR.

On the (+)strand of the RNA we find three long open triplet reading frames and three short open reading frames of 100-120 triplets. The (-)strand has no long open reading frames and eight short open reading frames of 100-145 triplets. The short open reading frames on both strands seem to be scattered randomly throughout the genome and none contains a candidate initiator ATG triplet near the 5' end of the open frame, thereby suggesting the short open reading frames may have no biological significance. The long open reading frames correspond to the *gag* gene (291-2,234), the *pol* gene (2,238-5,834) and the *env* gene (5,771-7,771) and are translated above the nucleotide sequence in Fig. 2.

The *gag* polypeptide

The *gag* (group specific antigen) gene of Mo-MuLV encodes a precursor polypeptide (Pr65^{gag}) of 65,000 daltons (based on SDS-gel mobility) which is cleaved into the four internal structural proteins of the virion²⁶. The four proteins have been designated p30, p15, p12 and p10 based on gel mobilities²⁷, and their order along the *gag* polypeptide is N-p15-p12-p30-p10-C (refs 26-30). The *gag* gene has been mapped as the most 5'-proximal gene²⁸. In this region, our sequence predicts an open reading frame at positions 291-2,234 which is terminated

by a single amber codon (Fig. 2). The first ATG triplet within this frame is at positions 621-623 and the use of this triplet as an initiator codon would produce a 538-amino acid polypeptide. Comparison of the predicted protein sequence with the available amino acid sequence data on the *gag* polypeptide and the individual *gag* proteins (ref. 31 and L. Henderson, T. Copeland and S. Oroszlan, personal communication) allowed positive identification of the predicted protein as the *gag* polypeptide.

For most eukaryotic mRNAs, translation is initiated at the ATG triplet nearest the 5' cap³². There are three ATG triplets between the 5' end of the RNA and our postulated initiator codon for the *gag* polypeptide. Each of the three triplets is followed closely by an in-frame termination triplet. Hence, completion of translation of the *gag* polypeptide initiated at any of these ATG triplets would require a splicing event. Alternatively, a splicing event may remove these triplets. The sequence AGGTAAG at 204-210 is a 7/7 match with the consensus 5' donor splice sequence compiled by Sharp³³. Joining this donor splice sequence to a possible 3' splice acceptor at 560-568 (6/7 match with consensus acceptor sequence³³) would remove the three ATG triplets and leave the ATG triplet at 621-623 as the most 5'-proximal ATG triplet on the mRNA. Thus, this splice would generate a 35S mRNA molecule with the

Fig. 2 (opposite) The sequence of a genomic clone of Mo-MuLV (pMLV-1) is presented as the sense (+)strand in the DNA form and represents the full-length RNA genome of 8,332 bases. The three long open reading frames are translated above the nucleotide sequence and their terminator triplets designated by asterisks. The known boundaries of the mature proteins are indicated by arrows. Features discussed explicitly in the text are underlined and they are (5' to 3'): the terminal redundancy (R), U₅, the inverted repeat correlated with transposition tRNA^{Pro} primer binding site, a 5' splice donor sequence, a possible 3' splice acceptor sequence for 35S mRNA, putative 3' splice acceptor sequence for the 21S mRNA, glycosylation sites of gp70, U₃, the inverted repeat and the terminal redundancy (R). The sequence presented here differs from the preliminary sequence distributed at the Cold Spring Harbor Tumor Virus meeting of May 1981, at positions 758, 893, 1,120, 1,168, 1,368, 1,868, 1,870, 1,922, 1,993, 2,018, 3,712, 4,306 and 7,970. Most differences were clerical errors and some were in regions that had been sequenced only once at that time. The protein sequence data of Oroszlan and colleagues presented at this meeting alerted us to possible compression artefacts in these regions and subsequent sequence determinations were carried out in conditions that minimized such artefacts. C. van Beveren's nucleic acid sequence data alerted us to two of the early errors. The construction and preliminary characteristics of the plasmid pMLV-1 have been described previously^{79,97}. Briefly, closed circular DNA molecules were isolated from clone 4A-infected NIH 3T3 cells, digested with the *Hind*III restriction enzyme (the Mo-MuLV genome has a single *Hind*III site) and cloned in *Hind*III-digested λ phage Charon 21A. The full-length genomic insert was subcloned from λ MLV-1 into the single *Hind*III site of pBR322 to generate pMLV-1. The 3' third (bases 4,895-8,332) of the insert is infectious; that is, a recombinant molecule with the 5' two-thirds derived from a clone of integrated Mo-MuLV and the 3' third derived from the genomic clone can transfect NIH 3T3 cells and produce infectious Moloney virus⁹⁷. The 5' two-thirds of the pMLV-1 insert have not been shown to be infectious. However, comparison of our sequence with the 3' two-thirds of the AKV sequence (W. Herr, personal communication) and with the available protein sequence data revealed that the *gag*, *pol* and *env* genes seem to be biologically correct. This reduces the region responsible for non-infectivity to the 5' copy of the long terminal repeat or to the first 600 bases of the RNA genome. We cannot explain the lack of infectivity of this cloned DNA. We determined the sequence of many of its restriction fragments by the partial chemical degradation technique⁹⁸. Our strategy involved sequencing all fragments except those which were clearly from within the vector and we later eliminated the vector sequences which were unintentionally obtained by a comparison with the pBR322 nucleotide sequence⁹⁹. The strategy was brute force until the few final regions, at which time we selected restriction fragments for sequence analysis. We assembled the fragments using the following strategy to optimize the accuracy of the final sequence. All autoradiographs were read independently by at least two of the authors, thereby minimizing 'reading' errors. All bases were seen on at least two independently generated sequence ladders, thus lessening the likelihood of gel and sequence chemistry artefacts. 95% of the final sequence was determined for both strands of the duplex DNA, thereby minimizing sequence-specific artefacts such as compressions. The sequences across all restriction enzyme cleavage sites were determined, thus eliminating the possibility that we would inadvertently introduce a deletion in the final sequence. However, as the viral genome was cloned in the *Hind*III site of pBR322, it was not possible to determine the sequence across the single *Hind*III site as it appears in the Mo-MuLV genome; however, because a very large, biologically correct, open reading frame extends across this site, we believe that our sequence is not lacking any bases in this region. The final sequence was extensively proofread for clerical errors, and as a final check, the printed sequence was compared directly with the primary data present on the autoradiographs.

proper structure to initiate synthesis of the *gag* polyprotein at the methionine codon at 621–623. Furthermore, such a splicing event may be involved in differentiating the two pools of 35S viral RNA found in cells—those found on polyribosomes from those packaged into virions³⁴.

Occasionally a larger, glycosylated form of the gag polyprotein is found on the surface of infected cells³⁵⁻³⁷ which has 4,000 to 6,000 daltons of additional peptide at its amino terminus³⁸⁻⁴⁰. Such a protein could be generated by splicing one of the ATG triplets at 254-256, 266-268 or 463-465 into the

gag gene open reading frame (291–2,234) before the postulated initiator ATG triplet for the unglycosylated *gag* polyprotein at 621–623. Note that the protein sequence translated from the nucleotide sequence of the *gag* gene open reading frame from bases 291 to 620 has several portions with similar features to the signal sequences of membrane proteins^{41,42}—a stretch of uncharged, mainly hydrophobic amino acids flanked by charged amino acids. Such a signal sequence might be involved in directing these *gag* polyproteins to the cell membrane. However, we find no compelling evidence in the nucleotide

[illegible][illegible]

sequence enabling us to predict the precise structure of a spliced RNA from which the glycosylated *gag* protein could be translated. Glycosylation involves attaching two high-mannose oligosaccharides to this larger *gag* polypeptide^{39,40}. One is attached near the amino terminus^{39,40}, probably at the glycosylation site (Asn-X-Thr/Ser) encoded by bases 693–701 of the genome; the other is attached in the p30 region of the polypeptide and we find four possible glycosylation sites in this region of the predicted sequence. By analogy to Rauscher murine leukaemia virus glycosylated *gag* polypeptide⁴⁰, the glycosylation site encoded by bases 1,794–1,802 of the genome is probably glycosylated. Presumably, glycosylation of some *gag* molecules and not others is a consequence of whether a signal sequence directs the particular ribosome to the endoplasmic reticulum.

Comparison of the predicted *gag* polypeptide sequence with the known amino and carboxy termini of the *gag* proteins³¹ revealed that the three cleavages required to produce the *gag* proteins are conservative. That is, in the primary sequence of the *gag* polypeptide, the carboxy-terminal amino acid of one protein is adjacent to the amino-terminal amino acid of the next protein. The precise sequence around each cleavage site appears unique (Fig. 3). However, two cleavages (p15–p12, p12–p30) are of the form aromatic amino acid–proline. The third cleavage (p30–p10) is within a short run of hydrophobic residues. The carboxy terminus of the predicted *gag* polypeptide is four amino acids longer than the carboxy terminus of p10, thus indicating that this region of the *gag* polypeptide also undergoes proteolytic processing. This site is similar to the p30–p10 cleavage site.

The individual *gag* proteins were named according to their apparent molecular weights on SDS-polyacrylamide gels²⁷. Their actual sizes can be determined from the predicted primary sequence of the *gag* polypeptide and the known cleavage sites. p15 has 131 amino acids (14,400 daltons), p12 has 84 amino acids (9,200 daltons), p30 has 263 amino acids (28,900 daltons) and p10 has 56 amino acids (6,160 daltons). The predicted protein sequences of the individual *gag* proteins are consistent with their biological roles^{43,44}. For example, the predicted sequence of the histone-like protein p10 is rich in arginine and lysine residues (14 of 56 residues). The major structural protein of the core shell p30 has two notable features. First, the number of basic residues is about equal to the number of acidic residues; possibly, salt bridges are involved in the stability of the core structure. Second, there is a sequence near the C-terminus of p30 that seems to have been tandemly duplicated. Although the nucleotide sequences are not obvious duplications, the amino acid sequences are quite conserved—Glu-Glu-Arg-Glu-Glu-Arg-Ile-Arg-Arg and Glu-Glu-Lys-Glu-Glu-Arg-Arg-Arg. The two differences are a Lys for an Arg and the insertion of an Ile residue. Possibly the nucleotide sequence was duplicated and subsequent genetic drift has blurred out evidence of the duplication at the nucleotide sequence level but not at the protein sequence level.

	Carboxy terminus	Amino terminus	
p15	arg-ser-ser-leu-tyr ---	pro-ala-leu-thr-pro	p12
p12	thr-ser-gln-ala-phe ---	pro-leu-arg-ala-gly	p30
p30	met-ser-lys-leu-leu ---	ala-thr-val-val-ser	p10
p10	gln-thr-ser-leu-leu ---	thr-leu-asp-asp	Pr65 ^{gag} C-terminus
Pr80 ^{env} signal	arg-gly-val-ser-thr ---	ala-ser-pro-gly-ser	gp70
gp70	asn-arg-his-lys-arg ---	glu-pro-val-ser-leu	p15E
p15E	val-val-gln-ala-leu ---	val-leu-thr-gln-gln	R-peptide

Fig. 3 The amino acid sequences around the processing sites in the *gag* and *env* polypeptides. The cleavage sites in the *gag* polypeptide were identified by comparing the predicted polypeptide sequence with the known amino- and carboxy-terminal amino acid sequences of the mature proteins³¹. The sites in the *env* polypeptide were determined similarly except that the C-terminus of Mo-MuLV gp70 has not been assigned.

The *pol* gene

The *pol* reading frame extends from positions 2,238 to 5,834 and is terminated by an ochre triplet. It is expressed as an 180,000-dalton protein (Pr180^{gag-pol}) that contains both *gag* and *pol* information^{45,46}. The ratio of Pr180^{gag-pol} to the *gag* polypeptide Pr65^{gag} in infected cells is between 1:100 and 1:10 (refs 46, 47). In the two models proposed to account for the structure and expression of Pr180^{gag-pol} (refs 46, 48), protein synthesis begins at the same initiator ATG triplet as for the *gag* polypeptide and proceeds through the *gag* coding region to the amber triplet that terminates the *gag* polypeptide. Both models then use an inefficient method of bypassing the terminator triplet to allow translation of the *pol* open reading frame and produce the appropriate ratio of Pr180^{gag-pol} to Pr65^{gag}. The first model involves suppression or readthrough of the terminator triplet and the second involves removal of the terminator triplet by an RNA splicing event.

The reading frames for the *gag* and *pol* genes are the same and are separated only by the single amber triplet that terminates the *gag* polypeptide (Fig. 4). Thus, suppression or readthrough of the amber triplet could generate Pr180^{gag-pol}. Indeed, the addition of an amber suppressor tRNA to a system that translates Mo-MuLV 35S RNA *in vitro* results in a decrease in intensity of the band identified as the *gag* polypeptide and a corresponding increase in an 180,000 dalton band containing *gag* and *pol* antigenic determinants^{49,50}. Hence, the relatively low amounts of Pr180^{gag-pol} found in infected cells could be due to the inefficient suppression of the amber triplet, possibly by a suppressor tRNA that is normally present at low levels in the cell. Alternatively, the amber terminator may be slightly leaky and as such readthrough could produce Pr180^{gag-pol}. The prokaryotic RNA bacteriophage Q β apparently uses a leaky UGA triplet to regulate the relative levels of its coat proteins⁵¹.

The second model involves removal of the terminator triplet for the *gag* polypeptide from the message by an RNA processing event. The RNA in the region of the *gag* polypeptide terminator might assume the secondary structure presented in Fig. 4. Such a stem-and-loop structure is reminiscent of the secondary structures seen at yeast tRNA processing sites⁵². Hence, an enzyme with specificity similar to that of a tRNA processing enzyme might recognize this structure as a substrate, cleave it and ligate the newly generated ends of the mRNA together. This process would remove the terminator triplet and could generate the large open reading frame necessary to encode Pr180^{gag-pol}. Such a mechanism or a conventional mRNA splicing event is most probably involved in the production of the *gag-pol* polypeptide in the avian sarcoma viruses because suppressor tRNA molecules have no effect on production of the *gag-pol* polypeptide *in vitro*⁴⁸. If such a splicing mechanism is used, it must be inefficient to generate the observed ratio of Pr180^{gag-pol} to Pr65^{gag}. As such, substrate selection errors by the tRNA processing enzymes, possibly induced by the structural mimicry to tRNA precursors, could account for the observed ratio.

The very large *pol* reading frame encodes 1,199 triplets or ~132,000 daltons of protein. The Mo-MuLV reverse transcriptase consists of a single polypeptide chain of about 80,000 daltons^{53–56}. Therefore, the *pol* region could encode another protein (or proteins) of up to 52,000 daltons. Because no protein sequence information is available on Mo-MuLV reverse transcriptase, the precise limits of the reverse transcriptase coding information remain undefined and hence the structure and size of the additional coding capacity are unknown. Furthermore, as 100,000 daltons of protein are sufficient to encode the *pol* functions in the avian retroviruses^{57–59}, the additional coding capacity of the Mo-MuLV *pol* gene is not necessarily involved in replication functions. Circumstantial evidence suggests that this additional coding capacity might encode a nuclease, by analogy to the avian RNA tumor virus system^{60,61}, or a protease involved in *gag* polypeptide cleavage⁶². We are trying to identify the various proteins in the same way we identified the carboxy terminus of the *env* polypeptide^{63,64}, that is, chemically

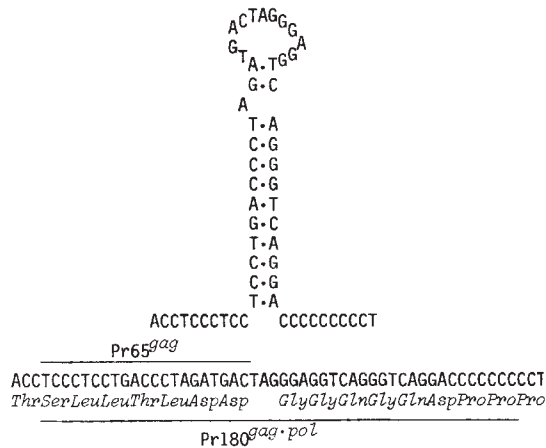


Fig. 4 The sequence about the *gag-pol* junction is presented as a linear sequence and a stem-and-loop structure. The C-terminal amino acid sequence of Pr65^{gag} is indicated above the nucleotide sequence and that of Pr65^{gag} and the hypothetical sequence of Pr180^{gag-pol} is translated below the nucleotide sequence.

synthesized peptides that correspond to portions of the predicted *pol* protein will be used to raise antibodies and the antibodies used to identify which protein(s) contain the antigenic determinants encoded by the peptides (reviewed in ref. 65).

The *env* gene

The *env* gene product is synthesized by membrane-bound ribosomes and is glycosylated as a nascent chain⁶⁶. The glycosylated polyprotein (Pr80^{env}) has an apparent molecular weight of 80,000 on SDS-polyacrylamide gels⁶⁶⁻⁷¹ and is processed in cellular membranes via proteolysis and carbohydrate modification to produce gp70, the major envelope glycoprotein, p15E, its membrane anchor and a small peptide R whose fate is unknown but whose cleavage seems to be involved in virus maturation or budding^{63,64,66-71}. (The R peptide does not correspond to the R region of the LTR.) Their order along the *env* polyprotein is N-gp70-p15E-R-C (refs 6, 72). The *env* polyprotein is translated from a 21S spliced mRNA which contains 200–600 bases derived from the 5' end of the genomic RNA and 2,500–2,800 bases from the 3' end of the genomic RNA⁷³⁻⁷⁷. This region of the genome contains one large open reading frame, extending from positions 5,771 to 7,771, which we have identified as the coding region for the *env* polyprotein by comparing the protein sequence predicted by this open frame with the experimentally determined protein sequences of the amino termini of gp70 and p15E (ref. 78 and L. Henderson, T. Copeland and S. Oroszlan, personal communication). Splice donor and acceptor sequences that could be used to generate the 21S mRNA are a 5' splice donor sequence at 204–210 (7/7 match with consensus acceptor sequence) and a 3' splice acceptor sequence at 5,493–5,501 (7/7 match with consensus donor sequence). As in the case of the *gag* mRNA, this splicing scheme brings the 5' end of the mRNA close to the open reading frame and removes all ATG triplets between the 5' cap of the mRNA and the first in-frame ATG triplet.

The putative initiator ATG triplet for the *env* polyprotein is at positions 5,777–5,779 near the beginning of the open frame. If

this ATG triplet is used to initiate the translation of the *env* polyprotein, the coding regions for the *pol* protein and the *env* polyprotein must be in different reading frames and overlap by 20 triplets. Interestingly, this ATG triplet has been postulated to be the initiator codon for the *src* protein of Moloney sarcoma virus⁷⁹, indicating a possible common route of expression for the *src* protein of Mo-MSV and the *env* polyprotein of Mo-MuLV. Furthermore, as the *src* gene substitution in Mo-MSV occurs at position 5,793 of the Mo-MuLV genome, the first five amino acids of the predicted *src* protein would be encoded by the residual 5' end of the *env* gene.

The *env* open reading frame encodes 665 amino acids or ~73,000 daltons of protein. The sequence of the first 26 amino-terminal residues of Mo-MuLV gp70 has been determined (L. Henderson, T. Copeland and S. Oroszlan, personal communication) and the sequence matches the predicted *env* polyprotein sequence beginning at residue 34. Therefore, the *env* polyprotein has a 33-amino acid leader sequence which resembles the 'signal sequences' found on other membrane protein precursors^{41,42}. For example, it contains a string of 11 hydrophobic amino acids that are flanked by positively charged amino acids. Because removal of these 33 amino acids from the *env* polyprotein would generate a protein of ~69,000 daltons which is consistent with the observed gel mobility (65–68,000 daltons) of the unglycosylated *env* polyprotein^{80,81}, the leader sequence is probably removed shortly after synthesis. By analogy to signal sequences, the function of such a leader sequence may be to direct the *env* polyprotein to the cell membrane and ultimately to the cell surface.

Glycosylation of the *env* polyprotein involves the addition of oligosaccharide chains to the gp70 portion of the nascent polyprotein⁶⁶⁻⁷¹. The predicted protein sequence of this region contains seven possible glycosylation sites of the form Asn-X-Thr/Ser. As gp70 molecules isolated from murine retroviruses carry six or seven oligosaccharide chains⁸², most or all of these sites are glycosylated. Furthermore, the two high-mannose oligosaccharides of Mo-MuLV gp70 are reported to be attached within the amino-terminal 250 amino acids of the gp70 protein⁸¹, thereby indicating that the glycosylation sites at amino acid residues 12–14 and 166–168 of gp70 are most probably involved in the attachment of these two oligosaccharides. The remaining five glycosylation sites are clustered at the C-terminal third of the gp70 molecule—within a region that is highly conserved between the Mo-MuLV gp70 and the AKV gp70 (W. Herr, personal communication) molecules. This region may be involved in the interspecies determinants found on the gp70 molecules^{83,84}.

The *env* polyprotein is cleaved during transit to the cell surface to produce gp70 and Pr15E (ref. 66). This cleavage, which is thought to occur in the lumen of the endoplasmic reticulum, seems to be generated by a protease with trypsin-like specificity, as the amino terminus of Pr15E is immediately preceded by a Lys-Arg doublet (Fig. 3). If this cleavage is typical of other secretory proteins with similar target sequences, such as insulin⁸⁵, then at least the two basic residues will be removed from the carboxy terminus of gp70. Pr15E is cleaved further to P15E and the R peptide at about the time of virus budding. This cleavage is within a sequence (Ala-Leu-Val-Leu) (A. Schultz, T. Copeland, L. Henderson, S. Oroszlan, personal com-

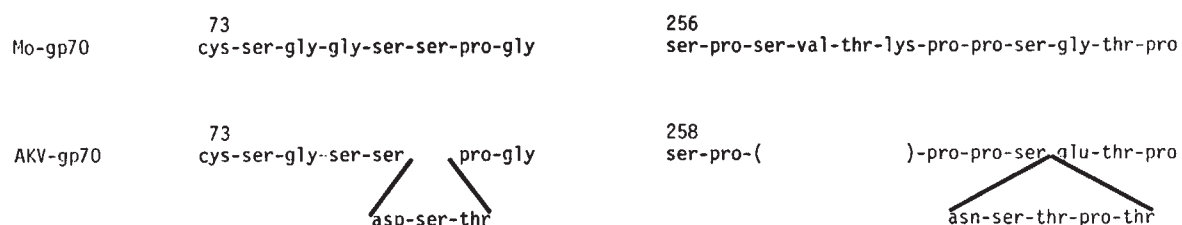


Fig. 5 Two regions of the Moloney gp70 sequence are aligned with the AKV-gp70 sequence (W. Herr and V. Corbin, personal communication). The numbers represent amino acid position in the protein chain, the parentheses indicate amino acids missing from AKV but present in Moloney gp70.

munication) that is similar to the sequences surrounding the cleavage sites in the gag polyprotein, thereby indicating a similar protease may be involved in the two cleavages. Perhaps R is cleaved in immature virions by the Pr65^{gag} protease coordinately with the gag polyprotein cleavage. Interestingly, the R amino acid sequence contains two differences with the R sequence obtained from a different Moloney clone⁶, perhaps indicating that this region undergoes rapid variation.

The two major cleavage products of the env polyprotein, gp70 and p15E, are disulphide linked⁶⁶. Because the gp70 molecule contains 20 cysteine residues and the p15E molecule 4 cysteines, it is not possible to predict which cysteine residues are actually involved in the disulphide bonds; however, it is tempting to infer that the cysteines at amino acid residues 72, 73 and 81 of gp70 interact with the cysteines at amino acid residues 86, 93 and 94 of p15E because both regions have the structure Cys-X₆ or γ -Cys-Cys. Both gp70 and p15E contain more than one stretch of uncharged predominantly hydrophobic amino acids which could span the cell or virus membrane.

The gp70 molecule is largely responsible for determining the host range of murine retroviruses and is highly polymorphic^{86,87}. The polymorphism is thought not only to reflect different viral strains but also somatic recombination, especially during the genesis of thymic neoplasias⁸⁸⁻⁹². Also, various differentiated immune lymphoid epithelial tissues express gp70 in the absence

of other viral proteins, suggesting that gp70 is a gene product of mouse not exclusively related to retroviruses⁹³⁻⁹⁶, perhaps involved in recognition. When we compared the gp70 sequence data of W. Herr and V. Corbin (unpublished) for murine retrovirus AKV with our Mo-MuLV sequence, we found that the AKV and Mo-MuLV gp70 sequences were colinear with occasional single amino acid substitutions, with two exceptions. These were two sites at which AKV gp70 had amino acid insertions relative to Mo-MuLV. At one of these sites Mo-MuLV gp70 had an insertion relative to AKV as well (shown in Fig. 5). Some of the gp70 polymorphism may be accounted for by variations at these two sites. Possibly they are hot spots for recombination or somatic mutation. Structural analysis of more gp70 proteins will be enlightening.

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LETTERS TO NATURE

A relationship between SNR G109.1-1.0 and the molecular cloud of Sh2-152?

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During a survey of the galactic plane at a frequency 0.610 GHz, Hughes *et al.*¹ discovered a new supernova remnant (SNR G109.1-1.0) which had already been detected as a radio source (CTB109) at 960 MHz by Wilson and Bolton². Optical observations by Hughes *et al.*¹ reveal filamentary structures, and a large [SII]/H α characteristic of SNRs has been measured by Blair and Kirschner³. Gregory and Fahlman⁴, using the Einstein X-ray Observatory, found at the position of the SNR an extraordinary new X-ray source. Their X-ray picture shows a clear semicircular arc with an angular diameter of 36 arc min which seems to be the outer shell of the SNR. Emerging from the east side of the central X-ray source GF2259+586 is a jet-like structure which curves northwards in another continuous arc smoothly joining the outer shell. From a number of common apparent features Gregory and Fahlman⁴ suspect another object of the same class as SS433. Here we report recent observations of the complex Sh2-147/Sh2-153 (see ref. 5) in the molecular line ^{13}CO ($J=1\rightarrow 0$) carried out with the newly developed POM telescope of the Bordeaux Observatory. Our results are compared with those of Israel⁶ in ^{12}CO . Several lines of evidence point to a physical association of SNR G109.1-1.0 and the X-ray source GF2259+586 with the molecular cloud of Sh2-152, as it has already been observed, for example, for IC443 and the Cygnus Loop⁷.

The millimetre wavelength observations were made in December 1980 and January and February 1981 with the 2.5-m antenna at the Bordeaux Observatory⁸. The ^{13}CO ($J=1\rightarrow 0$) line (110.20137 GHz) was observed. Half-power beamwidth was 5 arcmin and single-side band noise temperature $\sim 1,000$ K including atmospheric losses and emission. The receiver back end is composed of 256 channels, 100 kHz wide (0.27 km s^{-1} velocity resolution). Frequency switching by 5 MHz was used. ^{13}CO emission was observed at positions on a grid with a one-beamwidth spacing. The map of peak ^{13}CO equivalent brightness temperature (T_A^*) displayed in Fig. 1 shows close similarity with the CO map of Israel⁶ obtained at a lower resolution. The molecular cloud presents an elongated structure of about 1° long, corresponding to a linear dimension of 60 pc, if a distance of 3.5 kpc is assumed⁹. A low-brightness extension (δ) is detected north of the compact X-ray source. The two main maxima (α and β) are clearly associated with the H II regions Sh2-152/153 and Sh2-148/149. The line velocities range from -54 km s^{-1} at the SW end of the cloud to -49 km s^{-1} at the NE end (-47 km s^{-1} for the small cloud 40 arc min north of Sh2-152). The linewidths are moderately large ($2.5\text{--}4.5 \text{ km s}^{-1}$). A more detailed observational study of the cloud, including observations of other molecules, will be reported elsewhere.

We evaluated ^{13}CO column densities, using model calculations by Lucas¹⁰, and estimated the kinetic temperatures (T_k) from the ^{12}CO line intensities (as ^{13}CO lines are strong over most of the cloud, we may assume that the ^{12}CO lines are thermalized). At the position of Sh2-152/153, Dickinson *et al.*¹¹ found a brightness temperature maximum of 20K. We have

assumed $T_k = 20\text{K}$ at this position and $T_k = 10\text{K}$ in the rest of the cloud. In the direction of Sh2-152 we find $N(^{13}\text{CO}) = 3.6 \times 10^{16} \text{ cm}^{-2}$ for $n_{\text{H}_2} = 10^3 \text{ cm}^{-2}$, and $4.6 \times 10^{16} \text{ cm}^{-2}$ for $n_{\text{H}_2} = 10^4 \text{ cm}^{-3}$. Throughout most of the cloud $N(^{13}\text{CO})$ ranges from 2×10^{15} to 10^{16} cm^{-2} .

Assuming a $^{13}\text{CO}/\text{H}_2$ abundance ratio of 2×10^{-6} as proposed by Dickman¹² we may deduce molecular hydrogen column densities ranging from 10^{21} to $5 \times 10^{21} \text{ cm}^{-2}$ ($\sim 2 \times 10^{22} \text{ cm}^{-2}$ in the direction of Sh2-152). The total mass of the cloud may then be computed. We find values between 2×10^4 and $3 \times 10^4 \times (D/3.5 \text{ kpc})^2 M_\odot$.

Using different empirically determined relationships between radio surface brightness and diameter (the Σ - D relationship), Hughes *et al.*¹ and Gregory and Fahlman⁴ arrive at distances of 4.7 and 3.6 kpc respectively for the SNR. The distance 3.6 kpc is in fairly close agreement with the spectroscopic distance of the exciting star of Sh2-152 deduced by Crampton *et al.*¹³ and Pışmiş and Hasse¹⁴. However, distance determinations should be treated with caution, as kinematical distances derived for Sh2-152 as well as spectroscopic distances for other H II regions in the complex give different values. From the ^{13}CO observations we derive a kinematical distance of 4.1 kpc corresponding to $V_{\text{LSR}} = -50.0 \text{ km s}^{-1}$, if we use the rotation curve of Blitz *et al.*¹⁵

Figure 2 shows the positions of the 0.610-GHz radio map and the X-ray picture with respect to the molecular cloud ^{13}CO . There are two compact radio sources: B at nearly 7.5 arc min west to and A at about 10 arc min east to the centre of the radio structure. The radio structure is disymmetrical relative to B, and its centre is shifted eastwards by 5.7 arc min with respect to the

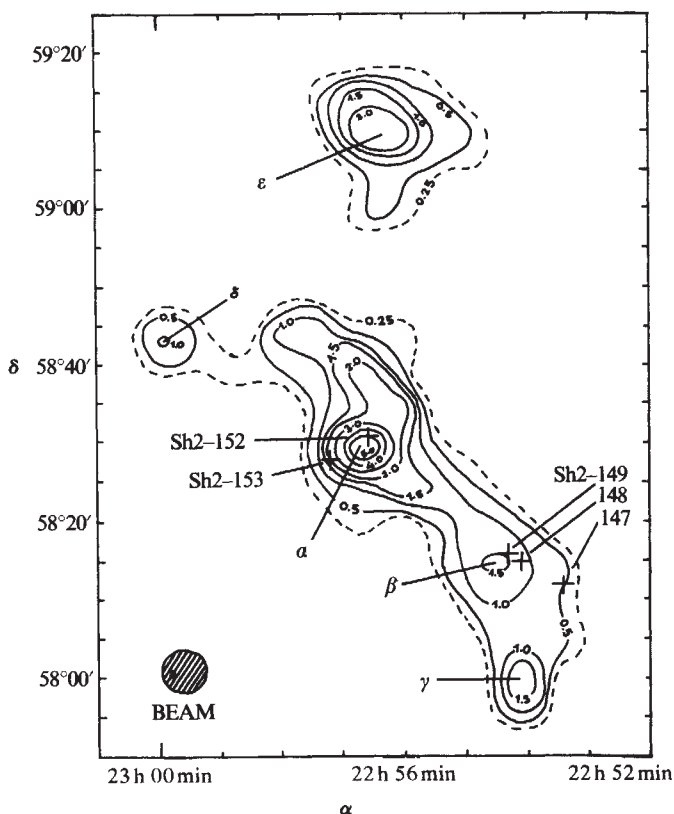


Fig. 1 Contours of T_A^* (^{13}CO , $J=1\rightarrow 0$) for the molecular cloud Sh2-147/Sh2-153 observed with the 2.5 m telescope at the Bordeaux Observatory. Crosses indicate the H II regions. Integration time was 15 min and half-power beamwidth 5 arc min. Greek letters indicate temperature peaks.

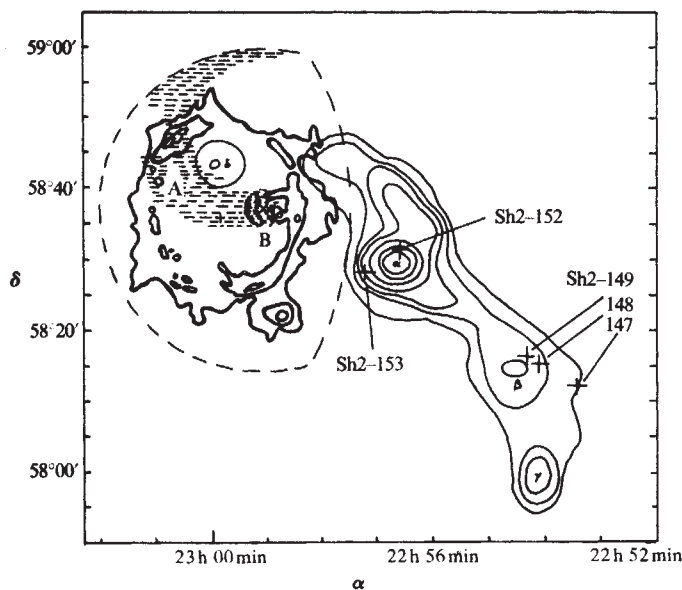


Fig. 2 SNR G109.1-1.0 superimposed on the molecular cloud. Thin line, contours of the ^{13}CO emission. Heavy line, 0.610 GHz radio contours of Hughes *et al.*¹, the centre of which is indicated by +; the compact radio sources A and B are also known. Dashed line, the extension of the X-ray emission. Hatched zone, X-ray jet. ×, X-ray compact source GF2259+586.

X-ray source GF 2259+586. There is also a 1.8 arc min distance between the X-ray source and B. The western edge of the radio structure is compressed, probably due to the collision between the blast waves of the SNR and the molecular cloud, as is its NW part near the peak δ .

The X-ray structure has a larger angular size than both the outermost boundaries of the radio structure and the optical filaments, in contrast to Cygnus Loop or the younger SNRs Cas A and Tycho for which the shell has nearly the same size in the X-ray and radio emissions.

An interesting feature of the X-ray picture is that it lacks emission in its western part, where the ^{13}CO emission is strong. Is the X-ray emission from the SNR being absorbed by the molecular cloud? The optical depth of the cloud for X rays in the energy range 0.1–4 keV may be deduced from the work of Ryter *et al.*¹⁶. A low-energy cutoff is present at ~ 0.35 keV for $N_{\text{H}_2} = 10^{21} \text{ cm}^{-2}$, 0.9 keV for $N_{\text{H}_2} = 10^{22} \text{ m}^{-2}$, ~ 1.5 keV for $N_{\text{H}_2} = 2 \times 10^{22} \text{ cm}^{-2}$ (while the interstellar absorption corresponding to $E(B-V) = 0.75$ in front of Sh2-153 would produce a cutoff at 0.7 keV). The central regions of the molecular cloud are then able to absorb almost all the X rays softer than ~ 1 keV, while the more diffuse parts of the cloud at its eastern edge will have radiation harder than 0.2 keV unaffected.

To go further we need precise information on the spectrum of the X-ray emission. If we assume a black body at $T = 3 \times 10^6$ K (corresponding to adiabatic expansion at a diameter of 40 pc, see Gorenstein and Tucker¹⁷), then most of the X-ray emission is below 1 keV, as expected for the X-ray spectrum of older SNRs. Then, the part of the X-ray emission lying behind Sh2-152 would be hidden.

The hypothesis that the X-ray emission has a low-energy component likely to be absorbed by the molecular cloud seems to be confirmed by the coincidence between the hole-like structure of the X-ray picture just north of the eastern jet and the low-brightness δ extension of the molecular cloud. On the other hand, the fact that the NE end of the molecular cloud has no apparent effect on the X-ray picture suggests that the molecular cloud is not effective in the absorption of X rays and that the radiation is emitted asymmetrically.

An earlier study¹⁸ indicates that Sh2-152 is not polluted by the products of nucleosynthesis of the supernova, but this may be due to the fact that the supernova explosion is a relatively

recent event, or perhaps that more quantitative methods are required to detect the contamination.

The various estimates of distance for the SNR, the X-ray source, the H II region Sh2-152 and the molecular cloud are in relatively good agreement, but because of the uncertainties of distance evaluation in the Perseus arm this cannot be regarded as conclusive proof of the association of these objects. The compression of 0.610 GHz radio structure at its western border adjacent to the molecular cloud, in addition to the apparent connection between the molecular peak δ and the NE radio concentration might suggest that the SNR is associated with the molecular complex. However, in the absence of observational data on the X-ray spectrum it cannot be firmly concluded that the dissymmetrical X-ray structure is due to molecular absorption.

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Zenith atmospheric attenuation measurements at millimetre and sub-millimetre wavelengths

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An accurate knowledge of the attenuation to be encountered over a vertical path through the atmosphere is vital for astronomy, communications and remote sensing in the millimetre region. We report here the results of spectroscopic sky emission measurements made at an altitude of 2.4 km from a mountain site (Izana) in Tenerife, Canary Islands, and compare them with predictions of a monomer water vapour model. An excess absorption is found which depends linearly on water vapour density with an approximate power law frequency dependence of 1.22; this dependence agrees with previous sea level measurements¹. We propose that line shape error is the cause of the excess and suggest a method for correcting astronomical observations for atmospheric attenuation and site testing.

The measurements were made as part of the site testing programme for the UK Millimetre Wave telescope project. Atmospheric emission was measured using a polarizing interferometer² with a helium-cooled (1.8 K) Ge composite bolometer over the range 100–1,000 GHz with a resolution of 9.6 GHz. Simultaneous measurements of precipitable water vapour were made using an IR hygrometer³ calibrated against

radio sonde ascents over an extended period⁴. The measurements were made with clear skies and a surface temperature of 22–28 °C during August 1980. Zenith precipitable water vapour (PWV) concentrations varied between 4.3 and 14 mm. Atmospheric emission was converted to opacity using an effective atmospheric temperature; full experimental details are given elsewhere (ref. 5 and C.C.Z., R. E. Hills and R. W. Barker, in preparation). Opacity measurements as a function of the zenith angle showed that the assumption of a plane parallel atmosphere holds for the site.

The discrepancy between the measured zenith sky attenuation in the millimetre window regions and the attenuation predicted by existing monomer theory is well known⁶. Excess attenuation has been seen from both sea-level and mountain sites in a wide range of conditions^{1,7}.

A multilayer computer model was used to compare the experimental data with monomer water vapour absorption. The model computes the oxygen and monomer water vapour attenuation in 30 layers from 2.4 to 74 km and is based on the AFGL line parameter tape⁸ and the Gross line shape expression⁹. The attenuation coefficient and line shape equation are as outlined by Falcone¹⁰. The computed spectrum is convolved with the SINC² function to account for the instrumental resolution.

Two empirical corrections have been used to account for the excess attenuation. The first one was obtained by Gaut and Reifenstein¹¹ from laboratory measurements, and has the form:

$$\Delta k(\nu) = 1.08 \times 10^{-11} \rho \left(\frac{300}{T_A} \right)^{2.1} \left(\frac{P_A}{1,000} \right) \nu^2 \text{ cm}^{-1} \quad (1)$$

where ρ is the monomer water vapour density in g m^{-3} ($1 \text{ g m}^{-3} = 1 \text{ mm PWV}$), ν the frequency in GHz, T_A the atmospheric temperature in K and P_A the total atmospheric pressure in mbar. The excess attenuation, ΔK , is given in units of cm^{-1} ($1 \text{ cm}^{-1} = 4.34 \times 10^5 \text{ dB km}^{-1}$) in accordance with previous papers. The second empirical correction was obtained by Rice and Ade¹ with data for a vertical path through the atmosphere from a sea-level site and is given by:

$$\Delta K(\nu) = 5.27 \times 10^{-10} \rho \left(\frac{280}{T_A} \right)^{2.1} \left(\frac{P_A}{850} \right) \nu^{1.22} \text{ cm}^{-1} \quad (2)$$

A pressure and temperature term has been added to the correction as given in ref. 1). The reference temperature (280K) and pressure (850 mbar) are for the water vapour scale height of the sea-level site used in the original measurements.

Comparisons between the measured attenuation and the model calculations were made at selected frequencies in the atmospheric windows up to 407 GHz. The higher-frequency windows were opaque over the precipitable water vapour range encountered. For each frequency, the predicted model attenuation was calculated with the Gross line shape alone, and with each of the above empirical corrections. Figure 1 compares the

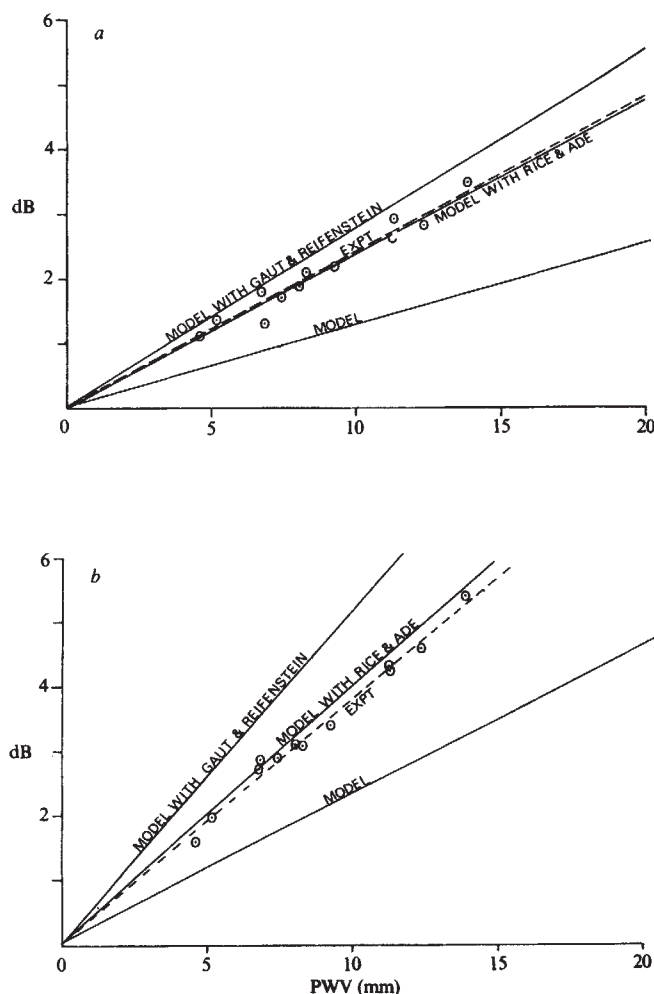


Fig. 1 Atmospheric attenuation as a function of precipitable water vapour for: a, 212 GHz; b, 287 GHz. The dashed line is fitted to the measured values. The lower solid line is the basic model with the Gross line shape only. The middle solid line is the model with the Rice and Ade¹ empirical correction. The upper solid line is the model with the Gaut and Reifenstein¹¹ correction.

attenuation with the precipitable water vapour at 212 and 287 GHz. The frequencies of 230 and 344 GHz were included because of their close proximity to molecular transitions of astronomical interest and are shown in Fig. 2. The values shown include the attenuation due to oxygen. Table 1 gives the exact frequencies and the experimental error of the mean attenuation for water vapour only. The predicted oxygen attenuation (Table 1, column 4) has been subtracted from the measured values. The experimental error involved with individual precipitable water

Table 1 Atmospheric attenuation

ν (GHz)	Experiment H ₂ O only (Neper mm ⁻¹ PWV)	H ₂ O only (dB mm ⁻¹ PWV)	% Error	Model O ₂ (dB)	Model H ₂ O only (dB mm ⁻¹ PWV)	Model H ₂ O only + Rice and Ade ¹ (dB/mm ⁻¹ PWV)	Model H ₂ O only + Gaut and Reifenstein ¹¹ (dB mm ⁻¹ PWV)
212.4	0.0551	0.239	±2.5	0.0204	0.125	0.240	0.276
229.63	0.0562	0.244	±2.1	0.0165	0.127	0.254	0.303
252.60	0.0647	0.281	±1.6	0.0126	0.154	0.297	0.368
287.04	0.0876	0.380	±1.4	0.008	0.228	0.394	0.504
344.45	0.187	0.813	±1.5	0.008	0.607	0.812	1.002
407.60	0.347	1.507	±3.8	0.147	1.202	1.455	1.757

Altitude, 2.4 km; ambient temperature, 25 ± 3 °C; resolution, 9.6 GHz.

vapour measurements is $<10\%$. Figure 3 shows the excess between the model and measured atmospheric attenuation for water vapour alone, together with the excess predicted by the two empirical corrections. The curvature of the empirical excess towards 407 GHz is due to a contribution from the sides of the atmospheric window, which appears because the empirical excess is taken from the model which includes the instrumental resolution.

Figures 1 and 2 show a linear dependence of attenuation on water vapour density. Figure 3 shows frequency dependence which approximates to a power law of 1.22. The expression of Rice and Ade is the better empirical correction for frequencies between 200 and 400 GHz. Two explanations for this excess have been proposed: that the excess is due to water vapour dimer absorption¹², and that the assumptions made about collisions in developing the collision-broadened line shape expression are not adequate to describe the far wings of a water vapour line⁶. The linear dependence on water vapour density observed here suggests that dimer absorption is not responsible for the excess, as a quadratic dependence would be expected. Furthermore, a linear pressure term in the Rice and Ade correction seems to account adequately for the altitude dependence. This is consistent with the excess being due to the inadequacy of the air-broadened water vapour line shape.

If the line shape error is the main cause for the excess window absorption between model and measured atmospheric attenuation, a possible explanation is as follows. The water vapour line

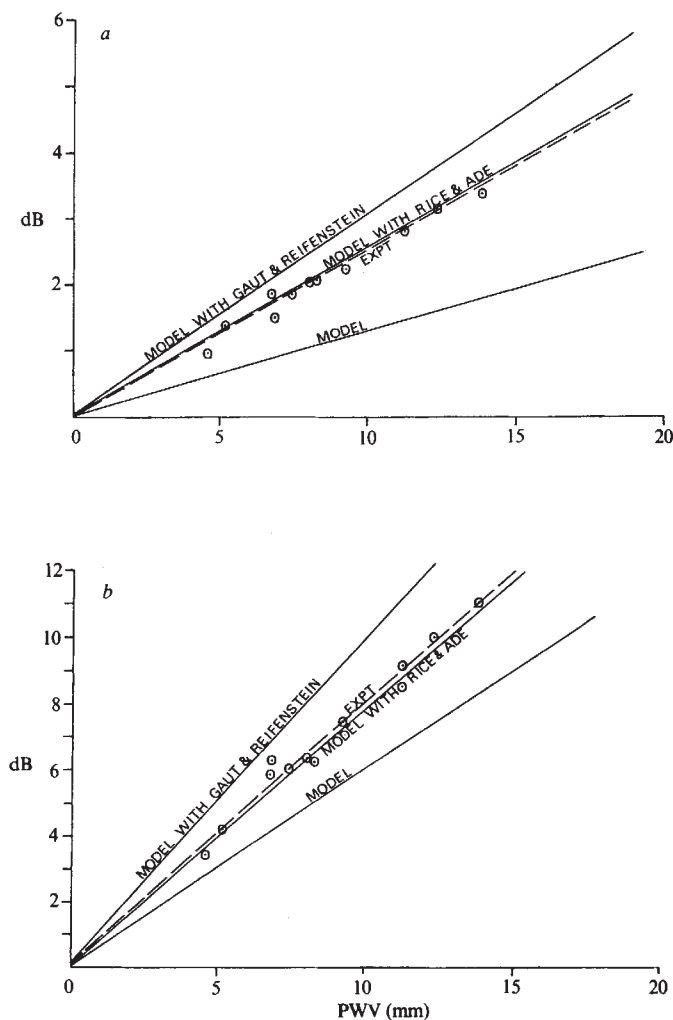


Fig. 2 Atmospheric attenuation as a function of precipitable water vapour for two frequencies of interest in astronomy: *a*, 230 GHz; *b*, 344 GHz. Model attenuation is as for Fig. 1.

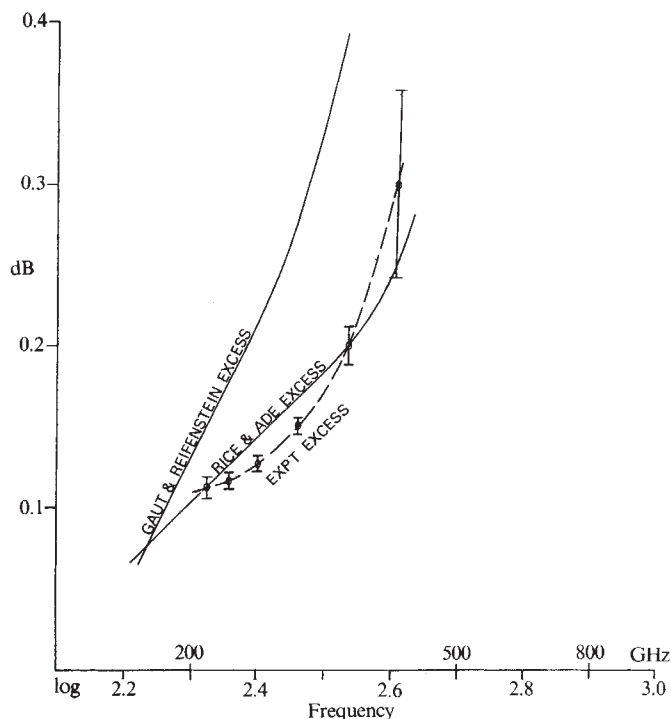


Fig. 3 The excess between the measured and basic model attenuation using the Gross line shape is shown as a dashed line. The model excess with the empirical corrections included in the model computation are shown by solid lines. The data points are taken from Table 1 and are for 1 mm of precipitable water vapour.

at 555 GHz (18.5 cm^{-1}) is stronger by a factor of 60 than any line below this frequency. The measured excess attenuation below 555 GHz shows a clear departure from a power law and could be interpreted as a line shape error of this line.

Further atmospheric measurements in the high-frequency windows should show whether the behaviour of the excess attenuation is consistent with our explanation. If line shape error is the cause of the excess, an empirical line shape can be used in a model to give the attenuation from a measurement of the precipitable water vapour in clear sky conditions. A water vapour meter based on a heterodyne radiometer is most suitable for such work. By using the emission on the side of 183.3-GHz water line, precipitable water vapour measurements can be made at any zenith angle during the day or night. This method can be used to correct astronomical observations for atmospheric absorption by measuring the precipitable water vapour over the slant path determined by the telescope zenith angle and direction. It has the advantages of not being affected by short-term variability, unlike existing methods, and gives attenuation in all atmospheric windows simultaneously.

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Peroxyacetyl nitrate in long-range transported polluted air

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The action of sunlight on air polluted with organic compounds and nitrogen oxides leads to a very complex mixture of reaction products^{1,2}. Peroxyacetyl nitrate (PAN) seems to be the most useful indicator of photochemically polluted air²⁻⁵ and was monitored during the summer of 1980 at Risø, a rural area, in Denmark and in Göteborg in Sweden (Fig. 1). These measurements seem to be the most northerly (56–58 °N) observations of PAN ever made. The long-range transport of ozone and of visibility-reducing aerosols has been observed on several occasions⁷⁻¹². In southern Scandinavia this is called a 'white episode'⁹. The measurements reported here reveal that high PAN levels occur during this type of episode. The comparison of these levels with those observed in optimal conditions for formation of PAN from local sources indicated that long-range transport contributes substantially to the presence of PAN in southern Scandinavia.

The atmospheric concentration of PAN was determined by electron capture gas chromatography^{3,5}. The experimental details will be described elsewhere¹³. Figure 1 shows a survey of the available data of other relevant species, and the positions of monitoring sites and meteorological stations.

PAN was monitored throughout the working day for 44 days between 11 June and 23 September 1980 at Risø. The average of the daily maximum levels was 0.9 ± 0.2 p.p.b. (parts per 10^9) (2σ). At the site in central Göteborg, the PAN concentrations were determined every hour between 17 July and 9 September 1980, except for 97 h. The average daily maximum level was 0.8 ± 0.2 p.p.b. Despite the distance between the two monitoring sites (230 km) and the great difference in the geographical surroundings, the PAN levels were close to each other and a significant correlation between the daily maximum levels was attained ($r = 0.72$; $p < 0.001$).

To evaluate the long-range transport component, the contributions from local sources must be established. Even on sunny days, air masses originating from central Russia and arriving at the Swedish west coast contain typically <70 p.p.b. ozone and low concentrations of particles¹⁴. Furthermore, the visibility range is high, suggesting low amounts of transported photochemical air pollutants. The position of Risø in relation to that of Copenhagen and Malmö, therefore presented an opportunity to observe PAN formation in the plume from a large city without serious interference of long-range transported photochemically polluted air. In fact, the distances from the two cities to Risø seem to be optimal for observing maximum PAN formation¹⁵. Two clear cases with high solar irradiation occurred. On 28 July and 30 July, the maximum PAN levels were 0.8 p.p.b. and 0.7 p.p.b. respectively (Table 1). The measuring site in Göteborg enabled another type of PAN episode to be observed from local sources. On two subsequent days, 13 and 14 August, a typical land/sea breeze situation with high solar irradiation occurred. On the first day, the maximum levels of PAN and ozone in Göteborg, and ozone in Rörvik were 1.0, 51 and 56 p.p.b. respectively. During the night, the ground-level concentrations decreased to the detection limits (PAN, 0.1 p.p.b.; ozone, ~5 p.p.b.). On the second day the concen-

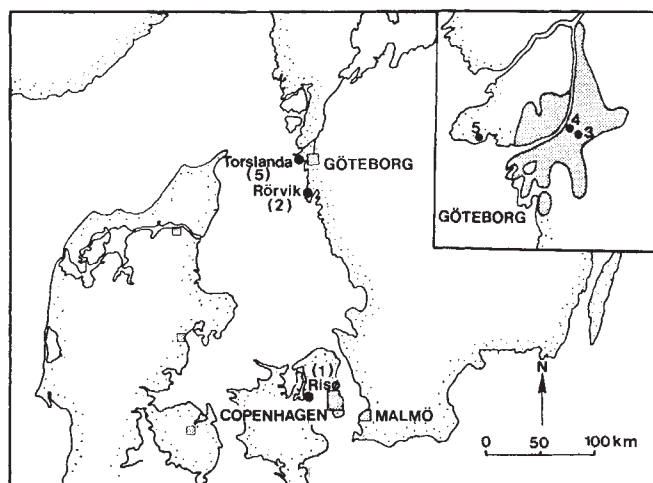


Fig. 1 Map of Denmark and west Sweden (inset: Göteborg) showing the positions of the monitoring sites and the meteorological stations (1), (2), (4) and (5). (1) and (3), monitoring of PAN. (2) and (4), monitoring of ozone, size-fractionated aerosols and "nitrogen dioxide" (the sum of NO_2 , PAN, organic nitrites and organic nitrates⁶).

trations increased to 1.7, 60 and 73 p.p.b. respectively. We conclude, therefore, that local sources may be able to build up substantial amounts of PAN only in situations with high solar irradiation and stagnant or recirculating air masses on subsequent days.

All cases with PAN levels exceeding 1 p.p.b. in Göteborg occurred during 13 August–9 September. The data from this period were, therefore, analysed more thoroughly to assess the importance of local and meso-scale formation and transport of PAN in relation to that of long-range transport. The diurnal variation of the average PAN levels was remarkably small. The average afternoon level was 0.62 p.p.b. and the average night-time level was 0.50 p.p.b. The production rate of PAN is much higher at daytime, and the lack of significant diurnal variation can only be explained if the dominant sources for PAN formation were situated more than 200 km from Göteborg. The wind-vector diagram (Fig. 2) shows that the highest concentrations of PAN are associated with ground-level winds coming from the south-west sector. The same pattern was observed in a corresponding diagram based on the directions of the daily air

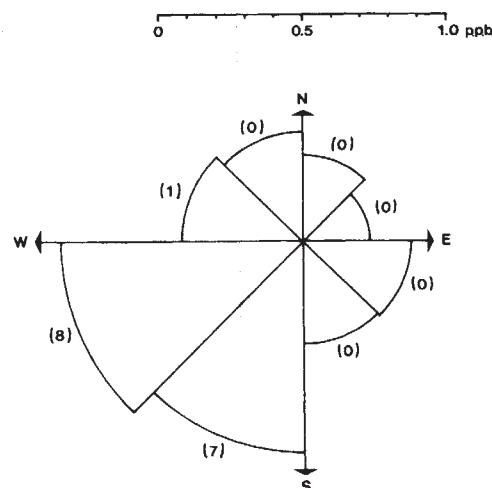


Fig. 2 Wind vector diagram for the average PAN concentrations in Göteborg between 13 August–9 September. Numbers in parentheses denote the percentage of cases with the concentrations on ≥ 2 p.p.b.

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Table 1 Comparison of three long-range transport episodes and cases representing the optimal conditions for observations of PAN formation from local sources

Type of episode Mean (a)	Max PAN conc. (p.p.b.)		Visibility range (km)	Mean ozone conc. (p.p.b.)		Mean particle conc. 0.4–0.6 μm (no. 1^{-1})	
	Göteborg	Risø	Göteborg 27	Göteborg 32	Rörvik 44	Göteborg 12,000	Rörvik 13,000 (j)
Long range (b)	2.8	3.2	7	48†	—	27,000†	31,300†
Long range (c)	3.5	4.2	5†	54†	76†	30,300†	34,200†
Long range (d)	2.1	1.2 (i)	11	41†	47	20,200†	27,000†
Land/sea breeze (e)	1.0		65	40	50	4,800	—
Land/sea breeze (f)	1.7		45	42	62	9,000	8,800
Transport from Copenhagen (g)		0.8					

(a) 17 July–9 September. (b) 28 August 13 MEST–29 August, 19 MEST. (c) 2 September, 22 MEST–4 September, 23 MEST. (d) 6 September, 5 MEST–9 September, 7 MEST. (e) 13 August, 11–19 MEST. (f) 14 August 12–17 MEST. (g) 28 and 30 July. (h) No PAN measurements 6 September, 14 MEST–8 September, 10 MEST (ozone maximum in Göteborg 8 September, 19–21 MEST). (i) No PAN measurements 6 and 7 September. (j) 7 August–9 September.

† Significantly different from the mean for 17 August–9 September ($P < 0.001$). MEST, middle European summer time.

mass trajectories, excluding the few cases with trajectories terminating only a small linear distance from Göteborg. A dominant part of the PAN transported into Göteborg originates, therefore, probably from distant sources in Great Britain and on the central continent. The high correlation between the PAN levels in Göteborg and those at Risø suggest that this is valid for the most of southern Scandinavia.

Between 28 August and 9 September, three 'white episodes' occurred. PAN levels exceeding 2 p.p.b. were observed for four days at Risø and for five days in Göteborg. In Table 1 the data are summarized and compared with the PAN levels in situations with favourable conditions for PAN formation from local sources at the two sites. Additional data available at the Göteborg site—ozone, particles and visibility range—are given.

The local meteorological conditions during these episodes were not essential to the PAN levels. Thus on 28 and 29 August (maximum PAN levels: 3.2 p.p.b. (Risø) and 2.8 p.p.b. (Göteborg)), less than 2 h of sunshine were observed at Risø and the cloud cover in Göteborg was almost constantly 100%. Furthermore, the highest PAN level in Göteborg in the first episode ((b) in Table 1) and in the second episode ((c) in Table 1 and Fig. 3) was observed at midnight, 2.8 and 3.5 p.p.b. respectively—this clearly excludes local sources. The wind structure during the three episodes was characterized by stable winds from the south-west sector and relatively high wind speeds at Risø and at the Swedish west coast ($\langle v \rangle = 8 \text{ m s}^{-1}$ at Rörvik). In view of the small formation of PAN in the plume from Copenhagen and Malmö, the contributions from cities in Denmark to the high levels observed in these episodes are considered negligible.

Figure 3 demonstrates the coincidence of the episodes in Göteborg and at Risø ((c) in Table 1). The relationship between

the concentrations of PAN and airborne particles in Göteborg, the flux of photochemically polluted air into the Swedish west coast and its effect on the visibility range are also borne out. The concentration of PAN in Göteborg correlated with that of ozone ($r = 0.76$; $P < 0.001$), aerosols ($0.4\text{--}0.6 \mu\text{m}$) ($r = 0.73$; $P < 0.001$) and 'nitrogen dioxide'⁶ at Rörvik ($r = 0.75$; $P < 0.001$), and with the visibility range ($r = -0.85$; $P < 0.001$). For the two other episodes (b) and (d) in Table 1, the data revealed similar relationships, although episode (d) was only partly characterized due to incomplete PAN measurements. The sources for formation of the high amounts of PAN are clearly the same as those giving ozone and visibility-reducing aerosols during the three episodes.

The 850-mbar trajectories for the air masses arriving at Risø and at Rörvik at 7 and 19 MEST (middle European summer time) showed that during the first two episodes ((b) and (c) in Table 1) the air masses came from England and/or the north-western part of the continent. The transport time over sea was ~25–30 h. On 5 September the air masses arriving at Rörvik had passed Scotland, while those arriving at Risø came from the continent. This may explain why no photochemical air pollution was observed in Göteborg and at Rörvik, while the maximum level of PAN at Risø was 2.0 p.p.b. (Fig. 3). During the third episode ((d) in Table 1) the air masses arriving at Risø and at Rörvik on 6 and 7 September and 8 September, 7 MEST, had passed England, and on 8 September, 19 MEST, and 9 September, 7 MEST, they came from the northwestern part of the continent mainly moving across land.

Increased PAN levels in polluted air masses which have been transported a few hundreds of kilometres have been reported^{4,16,17}. Our study of the presence of PAN in southern Scandinavia revealed that long-range transport (~1,000 km) of

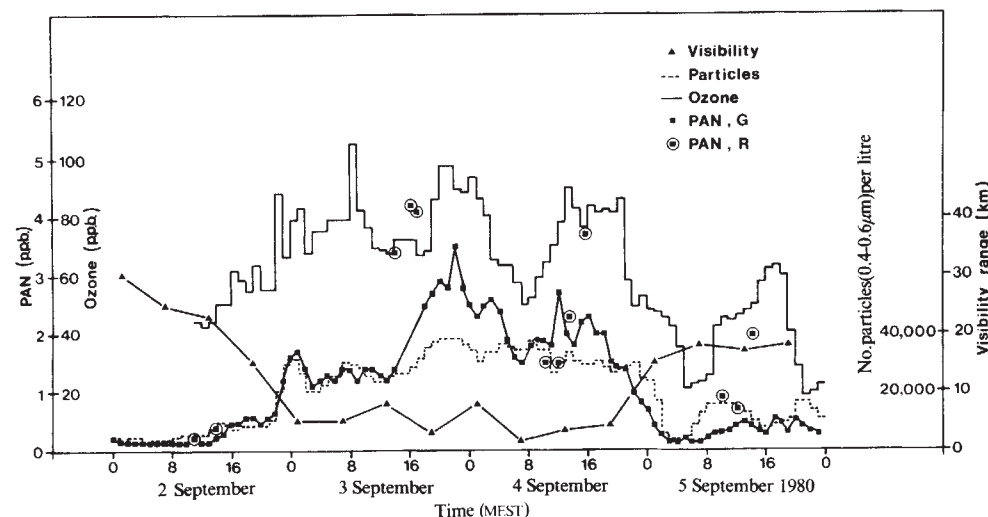


Fig. 3 The time variation of the concentration of PAN in Göteborg (G) and at Risø (R), the concentration of aerosols ($0.4\text{--}0.6 \mu\text{m}$) in Göteborg, the concentration of ozone at Rörvik and the visibility range at Torslanda before, during and after an episode of long-range transport (episode (c) in Table 1).

PAN occurs. During a more typical summer than that of 1980, the relative contribution of long-range transported PAN to the total amount of PAN may be less pronounced than observed in this investigation, but is probably still substantial.

The calculations of air mass trajectories were performed by the Norwegian Institute for Air Research and the Danish Meteorological Institute.

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Mercury emissions from Mount St Helens during September 1980

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Mercury emissions from active volcanoes are of interest for monitoring and forecasting volcanic activity, and for estimating the volcanogenic contribution of Hg to the atmosphere. We collected Hg from air samples during the first two weeks of September 1980, while Mt St Helens was in a non-eruptive phase. Mercury concentrations in the plume varied between 750 and 1,800 ng m⁻³. With these analyses we calculated that the daily Hg output from the Mt St Helens system ranged from 200 to 1,700 kg. Rough estimates of yearly Hg release from volcanic sources worldwide suggest that volcanoes may be important contributors of Hg to the atmosphere.

After the large eruption of 18 May 1980, the normal activity of Mt St Helens was limited to emission of a hazy plume of gas, rich in SO₂ and CO₂, which extended downwind from the volcano (ref. 1). Air samples were collected in this gas plume by aircraft, and ground-level air samples were collected in Vancouver (WA) for background determination². Mercury was collected by pumping unfiltered air over a gold-coated wire coil. The mercury was recovered from the collection coil by induction heating and analysed for Hg with an instrument (Jerome Instrument Co. Model 301, detection limit ~1 ng Hg in field conditions) based on the thin gold-film detection principle³. Flight traverses perpendicular to the plume were made ~15 km north-east of the crater at 100-m intervals in altitude (Fig. 1). Air was collected continuously during the traverses, and thus the samples contain Hg from both inside and outside the plume. The amount of Hg collected outside the plume (the ambient fraction) was determined with the aid of the CO₂ chart recordings (data from the United States Geological Survey [USGS]), which indicate the times spent inside and outside the plume (see Fig. 1). For the ambient Hg concentration we made measurements in Vancouver, upwind of the volcano, where we obtained an average value of 30 ng Hg m⁻³ (range 10-40 ng m⁻³). Having determined the amount of Hg collected inside the plume and calculated the volume of air sampled

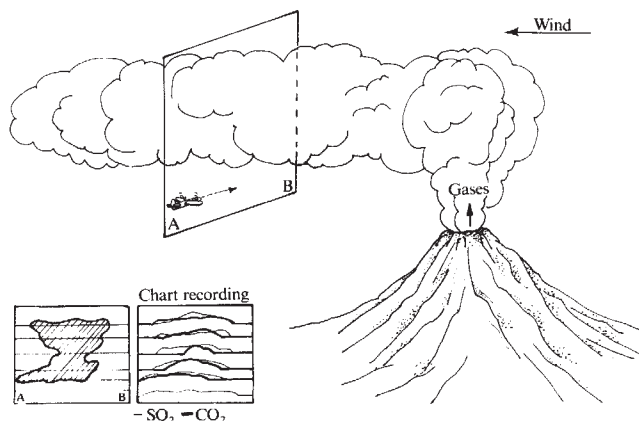


Fig. 1 Sketch of the geometric plane of sampling in the gas plume. Inset shows an example of SO₂-CO₂ chart recordings with a reconstructed cross-section of the plume. Horizontal lines represent flight paths through the plume at different elevations.

within the plume (again, from the CO₂ charts), we calculated the Hg concentration in the plume. The results are given in Table 1 and range from 700 to 1,800 ng Hg m⁻³.

Using the Hg concentration in the plume we can estimate the daily Hg output of the volcano. This output is calculated as the product of plume volume and Hg concentration in the plume (see formulae in Table 1). The daily plume volume is the product of the cross-sectional area of the plume and the average wind speed. The SO₂ and CO₂ recordings of the traverses through the plume (USGS data) were used to determine the shape and size of the cross-sectional area (Fig. 1).

The estimated daily Hg outputs are given in Table 1, together with SO₂ and CO₂ outputs (complete SO₂ and CO₂ data will be published elsewhere). The Hg outputs are rounded values, ranging from a few hundred kilograms per day to more than 1 tonne per day. There seems to be an initial trend towards higher outputs, followed by a sudden drop in output. A similar trend can be observed in the SO₂ and CO₂ data. There is an apparent correlation between the windspeed and these outputs, but over a longer period of time this is not generally the case for the gas data from Mt St Helens (W. I. Rose, personal communication).

Sources of error include collection efficiency and analysis, and extrapolation of the analytical data to the daily output estimates. The analytical error is <20% (ref. 4) and the collection efficiency adds only a few per cent to the total error; the error in the determination of the wind speed, the variation in the emission rate and non-uniformity of the emissions are difficult to

Table 1 Data used to calculate the Hg concentration in the plume, daily Hg outputs and results of these calculations

Days (September 1980)	5	6	8	10	15
A Total analysed Hg (ng)	8	10	9	21	13
B Hg from outside the plume (ng)	3	4	3	3	2
C Sampled volume of air from inside the plume (l)	6.5	8.4	5.8	9.9	9.5
D Hg concentration in the plume (ng Hg m ⁻³)	750	700	1,000	1,800	1,150
E† Average wind speed (km h ⁻¹)	25	46	37	4	3
F Daily plume volume (km ³)	1,350	2,000	1,700	450	200
G Hg output (tonnes day ⁻¹)	1.0	1.4	1.7	0.8	0.2
SO ₂ output (tonnes day ⁻¹)	600	1,300	1,800	300	400
CO ₂ output (tonnes day ⁻¹)	2,800	5,000	4,400	1,100	1,500
Hg/SO ₂ × 1,000	1.6	1.2	0.9	3	0.5

$$D = \frac{A - B}{C} \times 1,000 \quad G = F \times D.$$

CO₂-SO₂ data are from the USGS¹.

† Windspeed measured from the aircraft.

quantify. Relative errors in the wind speed determination can be large on calm days, up to 100%, but are small at wind speeds larger than 10 km h^{-1} . Although we believe that the magnitude of our results is correct, the numbers for the daily outputs are the result of extrapolations of analyses in the nanogramme range towards hundreds of kilogrammes.

Analyses for Hg adsorbed on the ashes from the major explosion of 18 May 1980 indicated Hg levels below the detection limit⁵. Analyses of the pumices erupted on 18 May gave values from 8 to 13 p.p.b. Hg (parts per 10^9), which are typical of the Hg concentrations in most volcanic rocks (refs 6, 7 and J.C.V., unpublished data), except for some unusual Russian occurrences⁸. Most of the Hg initially present in the magma seems to be released to the atmosphere before solidification of the magma. Thus Hg release patterns bear potential for eruption monitoring, such as signalling the influx of fresh magma into a volcanic system.

Studies of volcanogenic Hg output from Hawaii⁹⁻¹¹, Iceland¹² and Etna¹³ indicate that volcanic systems emit measurable quantities of Hg. Measurements of Hg adsorbed onto small particles from Etna gave outputs of 75 kg day^{-1} (ref. 13). The total output from Mt Etna is probably much larger because much of the Hg is present as vapour⁹⁻¹¹. Estimates of worldwide volcanic Hg fluxes differ significantly¹⁵. Unni *et al.*¹⁴ calculated a flux of $3-9 \text{ tonnes yr}^{-1}$. Anderson¹⁶, however, taking into account passively degassing volcanoes as well as active ones, calculated a release of $100 \text{ tonnes yr}^{-1}$.

Estimates of sources of atmospheric Hg have been given by McNeal and Rose¹⁷. They subdivide the sources into industry ($\sim 8,000 \text{ tonnes yr}^{-1}$), soil degassing ($\sim 9,000 \text{ tonnes yr}^{-1}$) and weathering of rocks ($\sim 2,000 \text{ tonnes yr}^{-1}$). However, according to Lantzy and MacKenzie¹⁸ these sources are insufficient to explain the observed relative abundance of Hg in the atmosphere. They invoked a Hg contribution from the oceans to explain this apparent shortfall, but data of Slemr *et al.*¹⁹ suggest that an oceanic source cannot account for the discrepancy.

It has been suggested that volcanoes should be considered as sizeable contributors to the world atmospheric Hg budget²⁰. If we apply our Hg/SO₂ ratio (10^{-3}) for Mt St Helens to the SO₂ flux data of Stoiber and Jepsen²¹, we arrive at a global flux of volcano-related Hg of $7,000 \text{ tonnes yr}^{-1}$. This estimate is based on a Hg/SO₂ ratio and SO₂ flux data from calc-alkaline volcanoes, which are the most numerous subaerial volcanoes in the world. As the current eruptive cycle at Mt St Helens started only a few months before our measurements, the magma is presumably still in the early stages of degassing. Thus, Hg/SO₂ ratios may be high relative to more mature systems. Also, the sudden emplacement of magma at shallow crustal level could have mobilized substantial amounts of Hg from the older volcanic rock series.

A Hg output from volcanoes between $1,000$ and $7,000 \text{ tonnes yr}^{-1}$ is our best estimate, taking into account uncertainties related to our data and calculations. This is in the range of other Hg contributions, and global budget calculations should consider volcanic systems as important sources of atmospheric Hg.

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Note added in proof: Preliminary data from Etna, Colima volcano (Mexico), and Hawaii indicate much lower Hg/SO₂ ratios (10^{-5} – 10^{-6}) than were obtained at Mt St Helens in 1980. Therefore, our worldwide volcanogenic Hg release result should be considered as a preliminary estimate.

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Energy straggling eliminated as a limitation to charge resolution of transmission detectors

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Detectors of the energy loss of penetrating charged particles are used extensively for particle identification. The chief limitation in these measurements is the fluctuation of the amount of energy deposited within the detector^{1,2}. We demonstrate here that this limitation can be overcome with a new nuclear track detector, CR-39(DOP), and that the charge resolution of this detector exceeds that of any other, including semiconductor diodes.

Because of their different response mechanisms, not all types of detectors are equally susceptible to energy loss fluctuations. This follows from the variance in the ΔE signal in a detector being proportional to ϵ_m , the maximum energy transfer to which a detector is sensitive^{1,2}. As the actual energy deposition depends on ϵ_m logarithmically, the fractional variance of ΔE is sensitive to ϵ_m . Ahlen¹ has reviewed the response mechanism of several particle detectors, and has classified them according to the type of atomic collision responsible for the detector signal. Ionization and semiconductor detectors are sensitive to all classes of collision, whereas, for high particle stopping power, organic scintillators respond preferentially to high-energy delta rays³. In contrast, dielectric track detectors are insensitive to primary energy transfers in excess of 10–350 eV (ref. 4). This has led to suggestions that dielectric track detectors should have greater charge-resolving capabilities than any other type of detector, including the semiconductor diode devices^{1,5}. Unfortunately, the poor quality of the plastic track detectors commonly used means that this could not previously be confirmed.

The recently discovered, highly sensitive CR-39 track detector⁵ has had two main drawbacks that had to be eliminated before we could demonstrate the superiority of track detectors over other transmission detection: (1) variations of sensitivity with depth and with surface position, and (2) development of a frosty surface after long etching times. The first problem prevents high resolution measurements due to large-scale inhomogeneities of the plastic structure. The second problem restricts the viewing to the top surface of a sheet of CR-39 and hence, to measuring at one time the parameters of only one of a series of etched cones associated with a particle track. We and Adams¹¹ have approached the first problem by considering the polymerization procedure used in the commercial production of sheets of CR-39. We thought that the cure time used in industry might be too short to allow uniform heat evolution and uniform polymerization required for track etch studies. After developing a heat transport model for a polymerizing sheet of CR-39, Adams¹¹ provided a commercial firm (Pershore Mouldings Ltd) with a temperature–time cycle thought to be appropriate for uniform polymerization. The resulting material was much more

uniform than previous samples. However, the CR-39 still turned frosty if more than $\sim 20\ \mu\text{m}$ of material were removed from a surface. This suggested that the polymer contained nonrandom structural inhomogeneities at roughly the scale size of the wavelength of light. However, within several centimetres of the edge of the sheets, which were in contact with vinyl gaskets during polymerization, the surfaces remained clear and smooth even if several hundred micrometres of material were etched away. One of us (G.T.) hypothesized that plasticizer in the vinyl gasket diffused into the CR-39 monomer and led to a more homogeneous polymer. He then showed that by deliberately adding about 1% of DOP (dioctyl phthalate, the plasticizer commonly used in vinyl gaskets) to CR-39 monomer, the entire sheet remained smooth and the etch-pits had good optical quality after prolonged etching. We believe that DOP causes this dramatic improvement by lodging between polymer chains so inhibiting formation of cross-linked clusters⁶. (An application for a patent for doped CR-39 has been filed with the United States Patent Office by G.T.)

To assess the charge resolution of the CR-39(DOP), which was made to our specifications (by Pershore Moulding Ltd), we performed a simple experiment at the LBL Bevalac. The configuration is displayed in the inset of Fig. 1. Ten sheets of CR-39 were positioned behind $7.77\ \text{g cm}^{-2}$ of polyethylene (CH_2) with their normal at an angle of 30° to the beam. Each sheet had dimensions $20 \times 10 \times 0.06\ \text{cm}^3$. The odd-numbered sheets (no. 1 being closest to the CH_2) had 1% DOP (by weight) added before polymerization. The even-numbered sheets had no additive. The primary beam, $1.86\ \text{GeV AMU}^{-1}\ ^{56}\text{Fe}$, uniformly irradiated the CH_2 and the CR-39 behind it with ~ 5 particles mm^{-2} . The CH_2 thickness was chosen to produce a significant amount of nuclear fragmentation of the primary beam with an allowably small velocity loss so that velocity dispersion effects could be neglected.

Following exposure, each sheet was etched for 1 month in a 6.25 M solution of NaOH at $40 \pm 0.01^\circ\text{C}$. Approximately $70\ \mu\text{m}$ of material was removed from each side of each sheet by general etching and the latent tracks of the iron and fragments were developed into conical etch pits, the projections on the residual surfaces of the sheets being ellipses.

The charge resolution of CR-39(DOP) was investigated by measuring the minor axes of track mouths in sheets 3 and 5. The sheets were aligned so that the four cones associated with a given particle could be observed through an optical microscope by focusing down through the sheets. Track dimensions were measured with a $\times 12$ eyepiece micrometer and a $\times 25$ air objective on a Leitz Ortholux microscope. For each track detected in the scanning process, successive measurements of minor axes for each of the four cone mouths were made and recorded. Note that with the non-DOP sheets their surface frostiness would have prevented seeing through the sheets. An area of $\sim 4 \times 5\ \text{cm}^2$ was scanned. Individual track mouths could be repeatedly measured with a single-measurement standard deviation of $0.1\text{--}0.15\ \mu\text{m}$. The average of the four minor axes obtained for each particle track is shown in Fig. 1. Note that of the 373 events tabulated not one has an ambiguous charge assignment. The charge resolution is $\sigma_Z = (0.118 \pm 0.006)e$.

These results can be used to determine the charge-changing cross-section $\text{Fe} \rightarrow \text{Mn}$ in CH_2 . Because there is only a very small difference in the total charge-changing cross-sections for Fe and Mn, then for our experimental configuration $N_{25}/N_{26} = nx\sigma_{25,26}$, where N_{25} and N_{26} are the numbers of observed Mn and Fe events, $n = n_C + n_H$ is the concentration of nuclei in the CH_2 , x is the CH_2 thickness and $\sigma_{25,26}$ is the $\text{Fe} \rightarrow \text{Mn}$ cross-section. The result is $\sigma_{25,26} = (111 \pm 22)\ \text{mbar}$. This compares favourably with the result of $(145 \pm 18)\ \text{mbar}$ obtained elsewhere⁷ with an identical primary beam and with a particle telescope consisting of six 3-mm-thick Si(Li) diode detectors. We suspect that our measured cross-section may be more accurate than that of Westfall *et al.*⁷ as the latter value seems to be on the high side of that predicted by fragmentation systematics and to be excessively greater than inferred from semi-

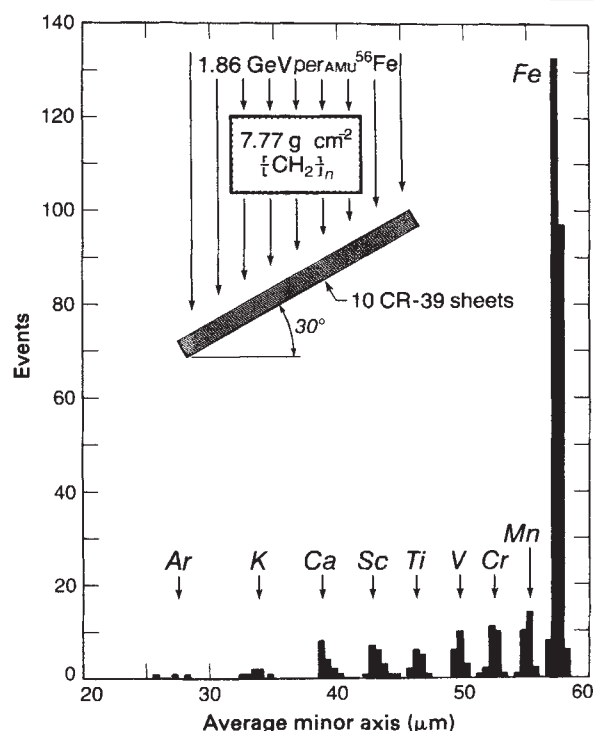


Fig. 1 Average minor axis of four track mouths in sheets 3 and 5. Inset shows experimental configuration for the irradiation.

empirical calculations (such deviations were not observed in ref. 7 for the $26 \rightarrow 24$, 23 , 22 cross-sections).

By applying standard relations on track geometry⁴, we have determined that the response of the top surface of sheet 3 can be characterized by a general rate of etching, v_G , of $0.10\ \mu\text{m h}^{-1}$ and by a charge-dependent track etch rate, v_T (for a velocity $\beta = 0.93$), of $s = v_T/v_G = 1.60(Z/26)^\alpha$ with $\alpha = 0.67 \pm 0.01$. This implies that CR-39(DOP) will record charges with Z as low as 13 for normal incidence with a velocity of $\beta = 0.93$.

We now compare the resolution of the CR-39(DOP) detector with that of a silicon diode detector. To do this we need to determine the effective thickness of CR-39(DOP) for a track mouth measurement. This thickness is smaller than the amount of material removed by general etching because the track mouth is causally related to only a fraction of the pathlength of the particle above the mouth. A least-time calculation⁴ shows that the effective thickness for a particle with zenith angle θ is given by $L_1 = v_G t s / (1 + s \cos \theta)$, t being the etch time. For the top of sheet 3, $L_1 = 47\ \mu\text{m}$ for $Z = 26$. For an equal number of g cm^{-2} , this corresponds to a silicon detector of thickness $26\ \mu\text{m}$. No one has directly measured the charge resolution of a Si detector of this thickness for $1.86\ \text{GeV per AMU Fe}$. We have, therefore, calculated this resolution; it has a standard deviation of $\sigma_Z(\text{Si}) = (0.52 \pm 0.05)e$. This calculation included fluctuations only of deposited energy. Fluctuations due to electronics noise and Fano fluctuations can be shown to be negligible. The error in σ_Z represents uncertainties due to assumptions internal to the model. This value of σ_Z is a factor of 2.2 times worse than that for an equivalent thickness of CR-39(DOP). If we use our same model to calculate the resolution for $1.86\ \text{GeV per AMU Fe}$ ions in 3-mm-thick Si detectors, we obtain $\sigma_Z = (0.15 \pm 0.02)e$ which agrees well with experimentally obtained values⁷ of $0.12e$ and $0.17e$. The value $0.12e$ corresponds to the configuration for which no matter is in front of the Si and the value $0.17e$ is for a Si detector placed behind the one with the better resolution. This difference is attributable to the showering of the second detector by delta rays produced in the first. Note that our calculation of Si resolution assumed the most favourable condition of having no matter in front of the detector. From actual measurements of resolution, we can say that the charge standard deviation of a CR-39(DOP) detector with a thickness equivalent to that of a

3-mm Si detector is $0.022e$, which is a factor more than 5 times better than the measured value for Si.

Four applications for this high-resolution detector are: (1) The exceptional resolution of CR-39(DOP) and the speed with which tracks can be processed make this an ideal detector for studying the anomalous properties that relativistic heavy nuclei seem to possess following nuclear interactions⁸. (2) The final frontiers of cosmic ray astrophysics involve accurate measurements of isotopic and elemental abundances of rare species for which both large collecting power and superb resolution are required. The extremely good charge resolution and low cost of CR-39(DOP) detectors make them suitable. (3) Past searches for primary antimatter in the cosmic rays have placed limits of ~ 1 part in 10^4 on the fraction of antimatter⁹. These limits could be improved by several orders of magnitude using a large-area array containing both plastic scintillators and track detectors. The track detector, which responds preferentially to distant collisions, measures the magnitude of particle charge, whereas the scintillator, which responds preferentially to close collisions³, can measure the sign of the charge by means of the sign-dependence of the Mott cross-section. (4) For a thickness of 5 mm of CR-39, a charge standard deviation as small as $0.02e$ is possible; this would enable the detection of non-integrally charged nuclei. Searches for energetic quarks bound to heavy normal nuclei are, therefore, made possible for studies either at accelerators or in cosmic rays¹⁰.

Studies of cosmic ray tracks show that the response of CR-39(DOP) is independent of track zenith angle and that the high resolution obtained in the accelerator experiment may also apply to the identification of heavy cosmic rays.

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Relict grains in chondrules

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It is not widely recognized that a significant fraction of the chondrules from ordinary chondrites contain silicate grains that survived the chondrule formation process without melting. In the most typical case these grains consist of coarse olivine, rarely orthopyroxene, crystals located in the core of chondrules and displaying a zoning that is inconsistent with crystallization from a silicate melt. The surrounding groundmass contains abundant glass and fine grained euhedral olivines that appear to be of igneous origin. The relict grains still preserve the imprint of processes that occurred in the solar nebula and, in some cases, may include the isotopic record of interstellar grains. The original properties of these chondrules are best preserved in the most unequilibrated ordinary chondrites^{1–3}. I present here important information with regard to the chondrule precursor materials and the process of chondrule formation which was acquired by a compositional and textural study of three of the most unequilibrated type 3 ordinary chondrites.

The spherical shapes and internal igneous textures of chondrules indicate that they were once molten. Although it is widely believed that chondrules were formed by melting of pre-existing solids^{4–8}, it has also been suggested that they are nebular condensates^{9–11}. The various textures observed in chondrules have generally been interpreted to result from differences in the rate of crystallization^{8,12}.

Models of chondrule formation date back to 1877 (ref. 13), but only recently has a substantial amount of analytical and textural data become available^{8,14,15}. The recent discovery of major differences in bulk and phase composition and oxygen isotope ratios^{8,15,16} in chondrules from unequilibrated chondrites indicate that a chondrite sample with centimetre dimensions contains a wide variety of materials formed in diverse conditions. Although Dodd^{6–17} identified in unequilibrated ordinary chondrites irregular rock fragments which have textural and chemical properties indicative of slow cooling and may represent the source rock from which chondrules were derived through shock, melting and chemical fractionation, it seems to have escaped attention that many droplet-like chondrules have never been completely molten, but preserve grains predating the chondrule formation event.

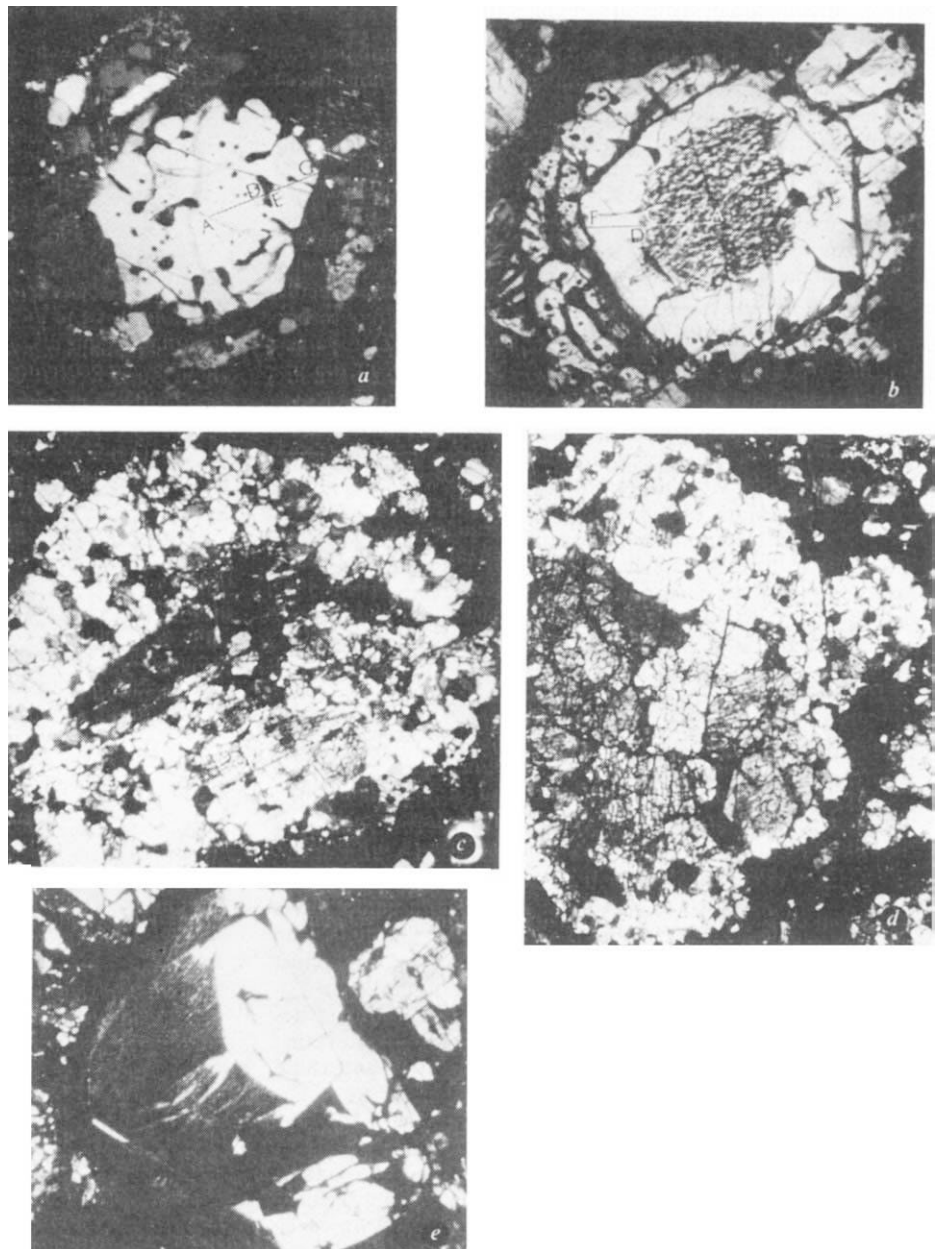
The most unequilibrated (type-3) ordinary chondrites have remained practically unaffected by post-agglomeration metamorphism and thus preserve numerous chemical and textural properties acquired during nebular processes^{1,3,18}. Sears *et al.*² used thermoluminescence sensitivity and other evidence to assign type-3 chondrites subtype numbers following the type number; the most unequilibrated type three is 3.0, the most equilibrated 3.9.

During a survey of chondrules in three of the most unequilibrated type-3 ordinary chondrites Krymka (LL3.0), Chainpur (LL3.3) and Bishunpur (L3.1), textural and chemical evidence of relict grains and thus incomplete melting was found. Some typical cases are discussed below. Independently, Nagahara¹⁹ has also reported the presence of relict grains in another unequilibrated chondrite Allan Hills A77015 (L3.5).

Chondrule A from Chainpur (Fig. 1a) consists of a central olivine grain that contains irregular olivine overgrowths and some interstitial silicate glass. Surrounding the large central olivine grain is a mixture of fine grained olivine, orthopyroxene and glass. Electron microprobe scanning from centre to edge of the large olivine yields the following results: the Fe content decreases by almost a factor of 6 from the grain centre to an intermediate area where glass inclusions first occur (A–D in Fig. 2, Ol 1 and Ol 2 in Table 1), then increases sharply by a factor of 20–30 in the outer portions of the olivine overgrowths (EF in Fig. 2, Ol 3 in Table 1). Manganese was found only in Ol 3. The smaller olivine grains in the portion of the chondrule outside the large grain also have a higher Fe content, with Fe increasing from centre to edge of each grain. The compositions of the external orthopyroxene and glass are also given in Table 1. The orthopyroxene is enstatite, and the composition is approximately that expected if in chemical equilibrium with the Fe-rich exterior olivine. The complex zonation of the large olivine is inconsistent with crystallization from a melt, which would produce olivines with Fe content monotonically increasing from centre to edge. This trend seems best interpreted to indicate that the olivine grain was never completely molten. Its inner portion (A–D in Fig. 2) might represent a relict grain from which the fayalitic component was partially lost by diffusion during a partial melting process. Alternatively, if there was too little time for equilibration, the inner portion (points A–C) may be a grain that initially contained $\sim 2\%$ Fe, and the lower Fe content of point D is the first olivine to precipitate from a melt that initially had a similar Fe/(Fe + Mg) ratio. Subsequent crystallization of olivine from the melt added the normally zoned overgrowth and the smaller olivine grains in the surrounding area.

Another case is shown by the Krymka B chondrule fragment (Fig. 1b). The large olivine grain in the interior has a dusty appearance, the dust consisting of submicrometre Fe–Ni metal inclusions; this region is surrounded by a clear rim. Olivine in the

Fig. 1 *a*, Chondrule A from Chainpur (diameter, 600 μm); the large olivine grain in the interior (white) has an inner core, delimited by droplet like or elongated glassy inclusions (dark), surrounded by olivine overgrowths which are in crystallographic continuity with the core. The outer portion of the chondrule consists of a mixture of fine grained olivine, orthopyroxene and interstitial CaAl-rich glass. *b*, Chondrule fragment B from Krymka (750 μm length); the large olivine grain has a clear rim but an inner dusty core due to the presence of fine grained metallic inclusions. Small spheroidal metal droplets are present outside the clear rim and droplets of SiO_2 -rich glass are included within the olivine (not visible in Fig.), the surrounding area consists of a mixture of olivine, orthopyroxene and glass of variable composition. *c*, In chondrule C from Bishunpur (2,000 $\times$ 2,650 μm) the inner portion consists of large dusty olivine laths, with rims free of metal inclusions. The outer portion consists of fine grained, euhedral olivines, Ca, Al-rich glass and spheroidal metal-sulphide droplets. *d*, Part of Bishunpur chondrule D (diameter, 2,750 μm) which consists of coarse grained enstatite in the core, surrounded by a mixture of euhedral olivine, Ca, Al-rich glass and spheroidal metal-sulphide. *e*, Chondrule E from Chainpur (diameter, 840 μm) contains large olivine grains partly grading into a fibrous mass consisting of an intergrowth of orthopyroxene and SiO_2 -rich glass.



dusty core (Ol 1 in Table 1) has higher Fe and Mn and lower Al and Ca contents than the clear rim (Ol 2). Olivine in the outermost portion of the rim is still richer in Fe (Ol 3). A compositional profile from centre to rim is shown in Fig. 2*b*. The material surrounding the olivine consists of an intergrowth of low-Fe olivine, poorly crystallized pyroxene (Table 1) and Ca, Al-rich glass of highly variable composition. Some small metallic droplets present outside the clear olivine rim are interpreted to have formed from dusty metal in regions that were completely melted. These chemical profiles and the preservation of the dusty metal in the interior seems inconsistent with growth of the host olivine from a melt. The detailed process will be discussed elsewhere²⁰. Reduction of fayalitic olivine to metallic Fe would have released SiO_2 , and excess SiO_2 is found in small glassy droplets in the clear olivine rim (Table 1).

Figure 1*c* shows a chondrule from Bishunpur (C in Table 1) consisting of large corroded dusty olivine laths, surrounded by thin clear rims. The outer portion of the chondrule consists of small, clear euhedral olivine grains, spheroidal metal and minor sulphide surrounded by Ca, Al-rich glass. No orthopyroxene was found. The dusty metal inclusions in the large olivines consist of almost pure Fe (>99% Fe), with only minor amounts of Cr, Co and Ni (refs 18, 20) which were probably produced by

in situ reduction of fayalite. Olivine in these dusty areas is difficult to analyse because of interference from the adjacent metal; occasional metal-free areas contains $\leq 2\%$ FeO (Ol 1 in Table 1) more Cr and Mn and less Ca and Al than the clear rims and the small euhedral olivine grains (Ol 2) in the surrounding groundmass. These euhedral olivines are slightly zoned with Fe increasing towards the edges. The occurrence of two types of olivine (large dusty laths and fine euhedral) displaying opposite zonal patterns is an indication of disequilibrium. It seems that the dusty olivine laths are relict grains that were never molten and predate the chondrule formation process. The reheating process was accompanied by reduction and formation of metallic Fe as in the case of chondrule B, followed by crystallization of Fe-poor rims and euhedral olivines from the melt. Small glass inclusions within the relict olivines (Gl 1 in Table 1) have compositions drastically different from that of the interstitial glass, being richer in Ca, Al, Ti, Mg, lower in Si, Fe, Cr and Na free. The composition of this glass is almost identical to that found by Fuchs *et al.*²¹ in aggregates from the CM Murchison chondrite. As discussed elsewhere^{18,22} Bishunpur C contains Cr, Si-bearing metal and Ca, Cr-bearing sulphide, an indication of high temperature origin in a reducing environment.

Bishunpur chondrule D consists of large twinned enstatite

grains surrounded by a mixture of euhedral olivines, spheroidal metal, sulphide, and Ca, Al-rich glass (Fig. 1d). The enstatite grains (Opx 1 in Table 1) have cores richer in Fe, Cr and Mn and poorer in Ca and Al than the rims (Opx 2). The euhedral olivines (Ol in Table 1) are slightly zoned with Fe increasing towards the grain edge; the glass (Gl) has high Ca and Al contents. This chondrule, as the previous one, contains Cr, Si-bearing metal and Ca, Cr-bearing sulphide^{18,22}. The opposite zonal pattern observed in orthopyroxene and olivine is an indication of disequilibrium and that some portions of the assemblage were never completely molten. No dusty metal was formed and the incomplete melting of the enstatite grains was followed by later crystallization of olivine in equilibrium with the Fe-poor enstatite rims. The excess FeO released during this crystallization may be responsible for the high values in the glass.

Chainpur chondrule E (Fig. 1e) consists of three large olivine crystals two of which appear to have partly grown into a fine-grained mass consisting of a mixture of fibrous pyroxene and SiO₂-rich glass of variable composition. The large size and the faceted appearance of the olivine indicate that these formed slowly at higher temperatures when diffusion was rapid, whereas the enstatite fibres seem to have formed during a short period at lower temperatures. Compositions of the three phases are listed in Table 1. The forsteritic olivine cannot have been in equilibrium with the SiO₂-rich glass. Complete crystallization of the chondrule system would have produced no SiO₂, but rather olivine and enstatite plus an accessory Ca, Al-bearing mineral. However, heating a forsterite-enstatite assemblage to the peritectic melting temperature of enstatite to produce a melt followed by kinetically favoured olivine crystallization as rims around the pre-existing unmolten olivine crystals could yield a melt rich in SiO₂. It is also possible that the material forming this chondrule originally consisted of a nonequilibrium mixture of coarse grained olivine together with a fine-grained mixture of pyroxene and SiO₂-rich mineral(s), and that rapid heating of this assemblage to the melting temperature of the fine grained mixture would leave the olivine unaltered and yield a product texturally and compositionally similar to that observed.

A survey of three thin sections, one for each of the chondrites studied (Bishunpur, Chainpur and Krymka) indicates that ~10% of chondrules or chondrule fragments show clear textural evidence of incomplete melting in which relict silicate grains predating the chondrule formation process are present. Electron microprobe studies of mineral assemblages inside some of these chondrules has provided the analytical data required to understand the chemical processes involved, and can probably be used to identify additional incompletely melted chondrules for which the textural evidence is ambiguous.

The identification of relict silicate grains in the interior of incompletely molten chondrules poses constraints on models of chondrule formation. The observations seem to exclude the possibility that these chondrules condensed as liquid droplets^{10,11}. We can make rough estimates of the maximum temperature achieved by these assemblages by treating them as equilibrium assemblages; the estimated temperatures in most cases must lie above the peritectic melting temperature of enstatite but below the melting temperature of pure forsterite. Of course, if the assemblage formed by flash heating, the instantaneous temperature of the melt could have been higher.

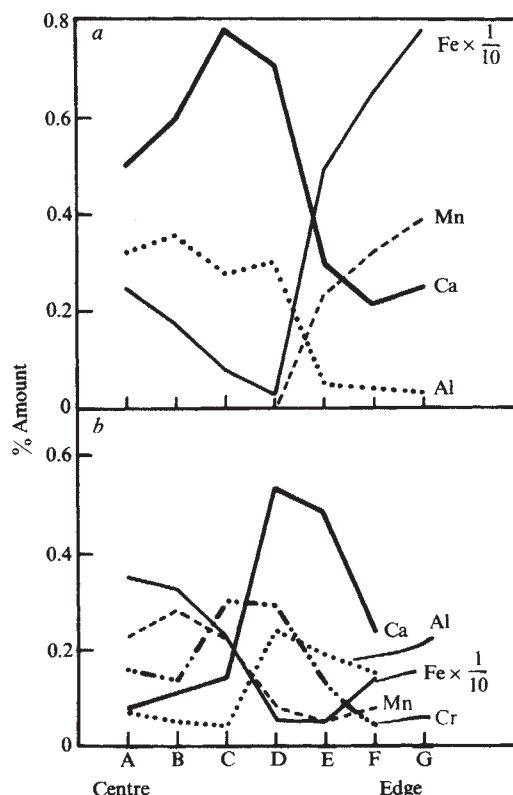


Fig. 2 Electron microprobe scanning from centre to outer rim of the large olivine grains in the interior of chondrules Chainpur A (a) and Krymka B (b). The complex zoning pattern indicates that the core of these grains never experienced melting.

In the case of chondrule D, the temperature to which the assemblage was heated must have been below the melting of pure enstatite, to allow the preservation of relict grains of this mineral.

After the reheating process, chondrules behaved as more-or-less closed systems, each constituting a specific chemical environment that was a function of the mineral assemblage and composition. An oxidized phase, possibly H₂O or CO, has been lost from the chondrule containing dusty metal, as some agent not now present seems to have been responsible for the reduction of FeO from fayalite to metallic Fe (ref. 20). Chondrules must have cooled rapidly enough to prevent internal equilibration by diffusion, allowing the preservation of zoning and disequilibrium assemblages in their interior.

The relict grains may be the product of nebular (or even presolar) condensation and their existence, not previously generally recognized in the chondrules of ordinary chondrites, offers a promising source of information concerning the chondrule formation processes and the precursor materials from which chondrules formed.

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Table 1 Chemical composition of coexisting silicate phases inside incompletely molten chondrules of highly unequilibrated ordinary chondrites

	Chainpur A					Krymka B					Bishunpur C				Bishunpur D				Chainpur E		
	Ol 1	Ol 2	Ol 3	Opx	Gl	Ol 1	Ol 2	Ol 3	Opx	Gl	Ol 1	Ol 2	Gl 1	Gl 2	Opx 1	Opx 2	Ol	Gl	Ol	Opx	Gl
SiO ₂	42.5	43.4	42.0	59.5	57.1	41.8	42.5	42.5	58.1	66.3	40.9	42.9	51.6	43.9	58.1	60.1	42.2	52.4	42.9	58.6	75.1
Al ₂ O ₃	0.35	0.26	0.03	0.27	20.5	0.07	0.26	0.04	2.5	22.1	0.03	0.23	22.9	30.2	0.24	0.54	0.05	22.5	0.05	0.59	9.2
MgO	55.3	56.7	51.0	37.1	3.5	55.6	56.7	56.9	36.2	4.3	55.0	55.9	4.7	5.7	36.0	37.9	54.5	4.7	54.4	35.1	9.3
FeO	1.74	0.31	7.5	3.3	2.4	3.4	0.62	1.43	0.75	2.0	1.54	0.78	1.2	0.58	4.6	0.62	2.2	3.8	3.2	3.0	1.9
CaO	0.59	0.71	0.25	0.25	11.0	0.10	0.54	0.24	1.0	1.3	0.20	0.46	13.4	17.4	0.27	0.44	0.25	11.8	0.14	0.70	3.5
MnO	0.01	0.01	0.31	0.21	0.23	0.25	0.05	0.06	0.34	0.03	0.27	0.05	0.05	0.03	0.27	0.08	0.10	0.15	0.21	0.46	0.31
Cr ₂ O ₃	0.11	0.08	0.13	0.68	0.27	0.15	0.17	0.05	0.65	0.85	0.19	0.38	0.49	0.32	0.55	0.28	0.12	0.45	0.10	0.78	0.39
Na ₂ O	—	—	—	—	5.0	—	—	—	—	1.8	—	—	4.2	0.01	—	—	—	3.2	—	0.12	0.58
TiO ₂	—	—	—	—	—	—	—	—	—	—	—	—	0.32	1.2	—	0.08	—	0.37	—	—	—
Total	100.6	101.5	101.2	101.3	100.0	101.3	100.8	101.2	99.5	98.7	98.3	100.5	99.5	99.3	100.0	100.0	99.4	99.4	101.0	99.3	100.3

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Late Palaeozoic palaeomagnetic data for Wrangellia: resolution of the polarity ambiguity

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Wrangellia and the Alaska Peninsula terrane are two of the many allochthonous terranes making up southern Alaska today. Palaeomagnetic data from these areas clearly indicate low palaeolatitudes in the early Mesozoic. Because of local and regional rotations, the mean declination of the palaeomagnetic vectors is uncertain. This, in addition to incomplete temporal coverage, has led to an ambiguity in the polarity of the vector, and hence an ambiguity in whether the palaeolatitudes determined are in the Northern or Southern Hemisphere. The data considered here have resolved the ambiguity for Wrangellia, and by implication for the Alaska Peninsula terrane, and also allow the construction of a highly speculative apparent polar wander curve.

A major portion of the North American Cordillera is composed of 'suspect' terranes^{1,2}. These tectonostratigraphic terranes are suspected of being allochthonous to cratonic North America. Palaeomagnetic studies have shown that many of these terranes have travelled great distances³; however, the geographic origin and tectonic history, in terms of plate motion, are still very poorly understood.

Palaeomagnetic data, particularly from severely deformed areas, are often difficult to interpret for use in palaeogeographical reconstructions. A palaeomagnetic pole may yield an estimate of the palaeolatitude and the amount of rotation necessary to reconcile any discordance between the ancient geomagnetic pole position and the present geographical pole, but gives no longitudinal control. There is also a polarity ambiguity which is particularly important at a low palaeolatitudes. As the Earth's magnetic field reverses polarity from time to time, it is not apparent whether the observed north seeking magnetic vectors correspond to the north geographical pole (normal polarity) or the south geographical pole (reversed polarity). The polarity will affect the sign of the palaeolatitude (Northern or Southern Hemisphere) and the amount of relative rotation needed to bring the palaeomagnetic pole into coincidence with the reference pole. Due to the sparse data base, especially with regard to time, the palaeomagnetic signatures of the terranes in Alaska are only poorly known; there are thus few guidelines to aid in the determination of the correct polarity. Consequently,

various workers have assigned polarities to minimize the required amounts of rotation, give continuity of motion and give reasonable plate velocities. These arguments are rarely convincing and the polarity dilemma has hampered construction of polar wander curves. There are two techniques to resolve the ambiguity of polarity. One is to construct a continuous apparent polar wander curve backwards in time from the present. This has not yet been possible for southern Alaska because of the difficulty of obtaining samples which have a wide enough distribution in time from appropriate locations. The construction of an apparent polar wander curve is also hampered by lack of precision in relating the measurements of the ancient declination of the geomagnetic field from one area to another. This difficulty arises from the fact that relative rotations of individual terranes may have occurred, as are seen elsewhere in Western North America³, and apparent local rotations may also arise through multiple folding events.

The second technique, which is applied here, is to use data from times when the geomagnetic field was anomalously stable. One of these times, the Permo–Carboniferous reversed magnetic interval, the geomagnetic field was almost exclusively of one polarity (reversed), times of normal polarity being both very short and extremely rare. The data discussed here are from rocks formed at the beginning of this period (when short normal polarity intervals were slightly more frequent) and in the middle (when they are extremely rare), thus allowing great confidence in our assumption of reversed polarity. We now report late Palaeozoic palaeomagnetic data of known polarity for the Wrangellia terrane⁴ and explore some of the ramifications for polar wander curves and tectonic history.

As originally described, the Wrangellia terrane⁴ comprises several tectonic units recognized in the Wrangell Mountains, Alaska, parts of southeastern Alaska, Queen Charlotte Island, Vancouver Island and an isolated fragment in eastern Oregon. Hillhouse⁵ presented palaeomagnetic data from Triassic rocks in the Wrangell Mountains which document low palaeolatitude, within 15° of the Equator. This low Triassic palaeolatitude has now been confirmed for other localities within Wrangellia^{6–9}. Hillhouse⁵ assigned normal polarity to the pole located at 146° E, 2° N on the basis that it is closer than the antipole to the equivalent Triassic pole for cratonic North America, and requires the lesser amount of northward translation and rotation for Wrangellia.

Three new localities have been sampled along Skolai Creek in the Wrangell Mountains, Alaska. These sample localities lie close to and stratigraphically beneath Hillhouse's⁵ Golden Horn Triassic locality (Fig. 1) and form part of the same, apparently intact stratigraphic sequence. The present results may thus be directly related to the Wrangellian Triassic palaeomagnetic poles, as these localities all seem to have experienced the same overall deformational history. Taking regional attitudes into account they may also be compared with cratonic North America.

Seventeen oriented cores were collected from both the Lower Permian Hasen Creek Formation (volcaniclastic sediments) and the Lower Permian or Pennsylvanian Station Creek Formation (volcanic, pyroclastic and volcaniclastic sedimentary rocks). In addition, six cores were obtained from a gabbro (Middle or Upper Triassic) intruding both the Hasen Creek and Station Creek Formations.

All samples were subjected to stepwise thermal demagnetization to remove secondary magnetic components. Primary vector directions were determined for each core on the basis of minimum change of direction over successive demagnetization steps, accompanied by systematic intensity decreases. Samples that did not display a stable vector direction for at least the last demagnetization steps were not used for calculation of a mean vector direction. In addition, vector directions more than an arbitrary 40° from the mean were eliminated from the calculation of the final mean on the basis that they probably represent either excursions of the geomagnetic field or errors incurred during the collection and processing of samples. Only

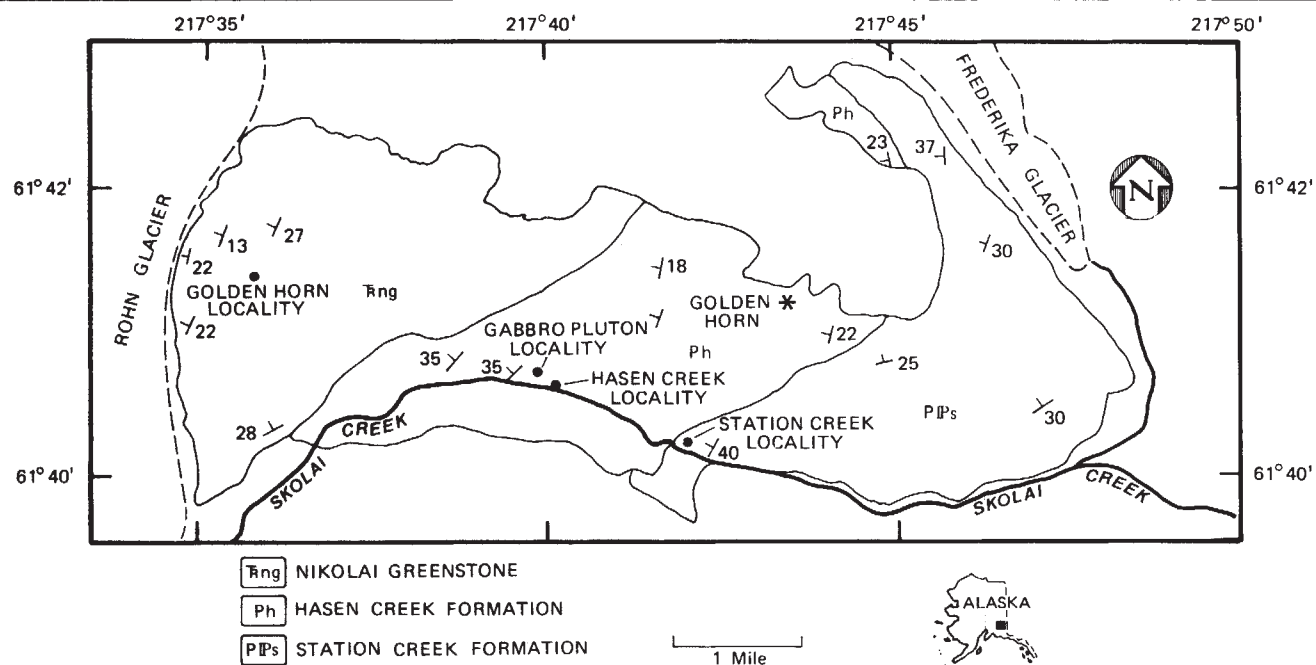


Fig. 1 Generalized geological map of Skolai Creek valley, eastern Wrangell Mountains, Alaska. Modified from ref. 16.

four vectors were rejected for these reasons (all from the Station Creek Formation). For all three localities, the mean vector directions with respect to present geographical coordinates do not include the present field within their circles of confidence. The fold test for these samples is positive, inasmuch as the precision parameter (k) (ref. 10) is larger when the data are calculated with respect to local indications of ancient horizontal than with respect to present horizontal. In addition, the Station Creek Formation suite contains one vector direction whose polarity is the opposite of the others. The mean directions (Table 1) thus represent a reliable measure of the ancient geomagnetic field.

The polarity for the virtual geomagnetic poles (VGP) can be assigned with confidence, as the Hasen Creek Formation is known from reliable fossil evidence to be of Permian age¹¹, which corresponds to the Permo-Carboniferous reversed magnetic interval¹². While there are rare normal magnetic intervals in this 'quiet' period, the probability of sampling only a normal interval in a thick (40 m) stratigraphic sequence is exceedingly small. Also, the existence of a single reversal in the earlier Pennsylvanian-Permian Station Creek suite is consistent with deposition within the section of the Permo-Carboniferous quiet interval which had a few short normal polarity episodes. Thus, taking into account the reversed geomagnetic polarity, the VGPs corresponding to the mean vectors plotted in Fig. 2 represent an estimate of the location of the north geographical pole for that time.

The VGPs for the Hasen Creek and Station Creek localities plot very close together. The VGP for the pluton is displaced towards the Triassic Wrangellian pole of Hillhouse⁵. Because a direct measure of stratigraphic tilt was not possible for the pluton, the tilt correction had to be assigned on the basis of local geology, and is thus inherently less accurate than tilt corrections for the layered rocks. However, the general position of the VGP for the pluton supports the validity of the other VGPs.

The VGP determinations reported here for the Pennsylvanian-Permian are located within 30° of the Triassic VGPs described by Hillhouse⁵. This provides strong evidence that Hillhouse's polarity preference is correct and that Wrangellia was located at lat. ~15° N, during late Pennsylvanian (?) to Triassic time.

Jones and Silberling¹³ suggest that Wrangellia and the Peninsular terrane had amalgamated by at least late Jurassic time, based on biostratigraphic considerations. If this interpretation is correct, then existing palaeomagnetic data for the Peninsular terrane should be applicable to Wrangellia from Jurassic time to the present. There is, however, a polarity ambiguity for the Jurassic Peninsular pole.

A Southern Hemisphere polarity for the Jurassic Peninsular pole has been proposed^{7,8} on the basis of 'smoothest' motion and minimum number of changes in direction of the inferred plate motion. The confirmed Triassic pole for Wrangellia is considerably closer to the Southern Hemisphere Jurassic pole for the Peninsular terrane than the Northern Hemisphere pole. Selec-

Table 1 Magnetic field data from formations in southern Alaska

Formation	Dg	Ig	k	Ds	Is	k	$\alpha 95$	Long. °N	VGP Lat.	k	$\alpha 95$	N
Hasen Creek (Lower Permian)	207.8	14.0	21.0	218.0	14.5	22.2	9.0	178.6	-14.9	41.3	6.6	11
Gabbro pluton	—	—	—	242.0	-2.8	111.0	5.4	152.1	-14.2	192.1	4.1	6
Station Creek (Lower Permian- Pennsylvanian)	215.4	1.8	3.1	225.9	10.9	8.9	14.9	167.6	-13.5	14.4	11.7	10

Dg, Ig are the declination and inclination of the magnetic vector with respect to geographical coordinates (present north and present horizontal); Ds, Is are the declination and inclination of the magnetic vector with respect to a stratigraphic frame of reference (present north and ancient horizontal); VGP is a virtual geomagnetic pole reported in terms of geographical longitude and latitude; k is Fisher's precision parameter; N is the number of samples. Geographical vectors for the pluton were not reported as all samples were assigned the same tilt correction; thus a fold test was not possible. The polarity of the tabulated data represent the equivalent of today's Northern Hemisphere pole.

tion of the Northern Hemisphere Jurassic pole as the correct one would also require $\sim 125^\circ$ of rotation. Similar rotations have been documented for numerous terranes³; however, these are considered to be related to regional shear in a transform setting. Evidence for similar transform tectonics for Wrangellia in Triassic or Jurassic time is lacking. On this basis the Southern Hemisphere Jurassic pole is favoured, which then allows a highly speculative polar wander curve to be drawn (Fig. 3) through the above Pennsylvanian–Permian data, the Triassic VGP of Hillhouse⁵ and the Jurassic, Cretaceous and Eocene VGPs of Stone¹⁴ and Stone and Packer¹⁵.

Because of possible significant errors in the declination data, due to unknown amounts of relative rotations of the two major terranes sampled, the highly speculative nature of the polar wander curve must be emphasized. Even taking this into account, the apparent polar wander path shows two pronounced 'kinks': one in Jurassic time and one some time between late Cretaceous and Eocene time. The possibility that these kinks represent changes in the geological regime is enhanced by the fact that the Jurassic aged 'kink' also reflects a change in the trend of the palaeolatitude versus time of the sampling sites. Because the palaeolatitude depends solely on the inclination of the magnetic vector with respect to ancient horizontal, it is unaffected by relative rotation. The timing of these sudden changes of trend in tectonic motion with respect to the geomagnetic, and by implication the geographical pole, are reasonably close in age to two established orogenic episodes in Alaskan geology. We speculate that the Jurassic orogenic event marks the amalgamation of Wrangellia and other southern Alaska terranes, probably including the Peninsular terrane. The later event marks the accretion of this superterrane to North America some time during late Cretaceous to Eocene time. In this model, the kinks in the polar wander path would be the palaeomagnetic signatures of the collision events, which in turn caused readjustments of the motions of the terranes.

If this polar wander path is correct, it also implies that Wrangellia, and whatever plate it was part of, had significant southerly motion during earliest Mesozoic times. This was then followed by northerly plate motion beginning in the Jurassic period which carried Wrangellia and the Peninsular terrane

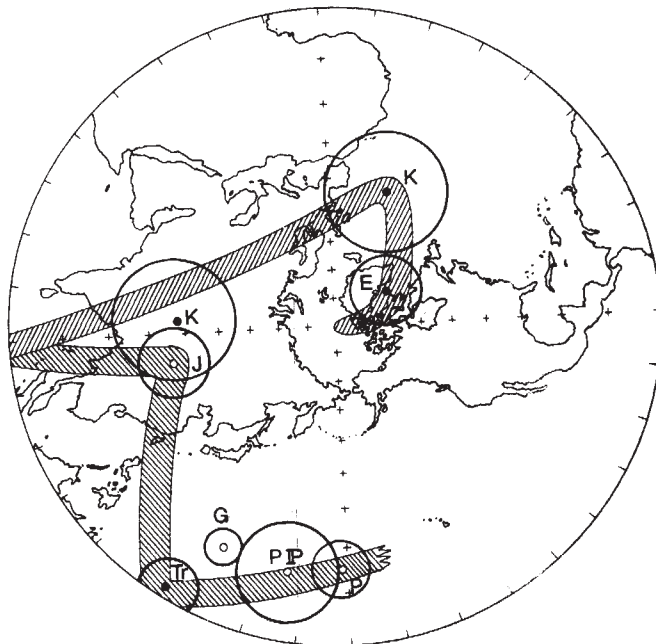


Fig. 3 Speculative polar wander curve for Wrangellia. Permian–Pennsylvanian and Triassic poles are considered to be fixed, inasmuch as the choice of polarity is almost certainly correct. Jurassic to Eocene poles are from the Peninsular terrane, based on the assumption that the Peninsular terrane and Wrangellia were joined by late Jurassic. ●, Northern Hemisphere; ○, Southern Hemisphere.

from the Southern Hemisphere to their present location in southern Alaska.

Obviously we cannot calculate actual rates of plate motion from the data available, but the data do not require excessive motions. The maximum plate velocity needed is $\sim 6 \text{ cm yr}^{-1}$ of latitudinal movement between the Jurassic and the present. In principle, it may be possible to make generalized reconstructions of the motions of the Palaeo-Pacific oceanic plate(s) by using allochthonous terranes as 'tracers'. This is an important possibility, as there is no Pacific oceanic crust older than Jurassic from which plate motions can be ascertained directly.

Although more sample localities are desirable to establish firmly the position of the Pennsylvanian–Permian poles, the evidence confirms the polarity of Hillhouse's⁵ Triassic palaeomagnetic pole for Wrangellia. This resolves the polarity dilemma for part of the Wrangellia polar wander curve, and will reduce polarity problems in future studies.

The highly speculative polar wander curve for Wrangellia, based on data from both Wrangellia and the adjacent Peninsular terrane, can also be verified (or refuted) by more sampling, particularly in the time-stratigraphic intervals in Wrangellia which currently have sparse or marginal palaeomagnetic data.

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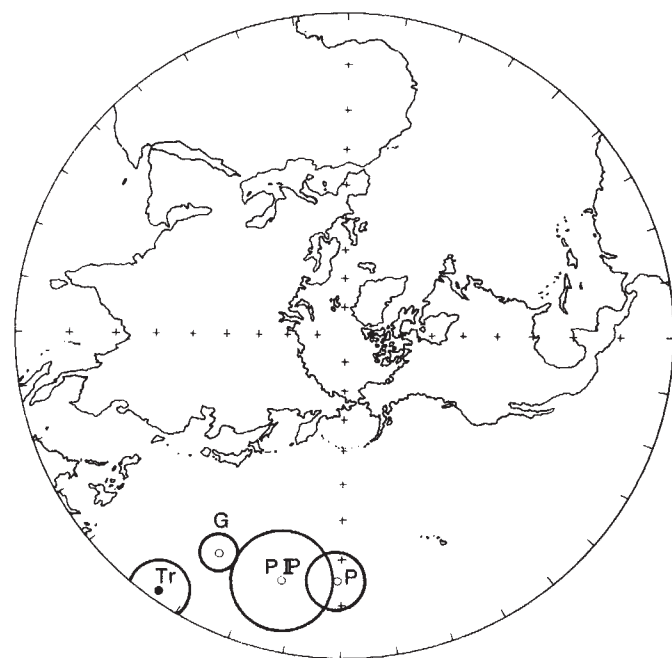


Fig. 2 Virtual geomagnetic pole (VGP) positions and associated circles of 95% confidence for upper Palaeozoic units. P, Hasen Creek Formation (Permian); PP, Station Creek Formation (Permian–Pennsylvanian); G, a gabbro pluton (Triassic). ●, Northern Hemisphere; ○, Southern Hemisphere.

Intermediate levels of soil disturbance maximize alpine plant diversity

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The intermediate-disturbance hypothesis¹⁻⁹ predicts that plant species diversity will be maximized at intermediate levels of vegetation disturbance (D). As yet, the only directly applicable data seem to be from Grime's¹ study, which served more as a source of the conjecture than an independent test of it, and in which disturbance was not measured. Here I report an analysis of the relationship between soil frost disturbance and diversity of vascular plant species in Arctic-alpine fellfield vegetation in the White Mountains of interior Alaska. To study plant species diversity, a measure (SD) is calculated, which is affected by both the number of species (species richness, SR) and the evenness of their relative abundances. I show that intermediate levels of disturbance maximize SD by influencing the evenness of relative abundances, but not the overall number of species. Because disturbances caused by soil frost action and animals are frequent in many Arctic and alpine tundras, these results imply that disturbance could be an important factor in species diversity differences within and between tundra vegetation types. If theoretical predictions¹⁻⁹ are confirmed by dynamic tests now under way, the theory could be applied to problems of tundra vegetation management such as revegetation and disturbance by trampling and vehicles.

The argument of the intermediate-disturbance hypothesis is, briefly, that disturbed patches will host a local succession of species during their post-disturbance recovery, that is, they will undergo gap-phase or micro-succession⁴⁻¹². Thus in areas sufficiently large to contain several patches, at intermediate levels of disturbance the vegetation would contain a relatively equitable mixture of differently aged patches in various stages of recovery, and therefore a relatively even mixture and greater variety of species (including early- and late-successional plants). A test could follow one of two approaches: (1) verifying the predicted¹⁻³ SD - D relationship at a 'macroscopic' scale, or (2) demonstrating microsuccessional dynamics at the patch scale. The former approach is used here.

The study sites are 136 and 169 km north-east of Fairbanks, Alaska, along the Steese Highway, at an elevation of 950-1,100 m. The fellfields occur on windswept ridges or slopes bearing little or no permanent snow cover; the discontinuous, very low (1-5 cm) vegetation is dominated by a few species of creeping perennial subshrubs, mainly *Dryas octopetala octopetala*, *Diapensia lapponica* and *Salix phlebophylla*, and by fruticose lichens (for authorities on nomenclature, see refs 13, 14). Between vegetated patches, wind erosion has exposed a discontinuous stony pavement set in a matrix of sandy to silty loam soil. The soil annually undergoes a number of freeze-thaw cycles, creating small (0.2-1.0 m) frost scars and frost-heaving some plants. This mosaic of exposed pavement, lichen patches, vascular plants and frost scars is probably dynamically inter-related by microsuccessional sequences (see refs 15-19; see ref. 19 for an illustrated introduction to the relationship between alpine soil frost disturbance and vegetation). In highly frost-disturbed areas, caespitose and rhizomatous perennial herbs in *Arenaria*, *Oxytropis*, *Campanula* and *Antennaria* are frequent; in relatively undisturbed areas, these species contribute little cover compared with subshrubs.

Species diversity (SD) was estimated^{20,21} as $1/(\sum p_i^2)$, using replicated ($N = 2-7$), 5 m line transects set 0.5 m apart, in which species cover was measured by point intercepts at 1 or 2 cm intervals; p_i is the proportion of total vascular plant cover contributed by species i . Ranked species-abundance curves based on the p_i are geometric^{20,21}. Vascular plant species rich-

ness (SR) is the number of species recorded in each transect of 250-500 points. 'Disturbance' (D) is estimated as the proportion of a line transect covered by recently frost-churned soil, which is unvegetated and yellowish, with no dark algal crust. Note that D is not a direct measure of disturbance rate. A test of the hypothesis will, nevertheless, be valid if the intersite ranking of D concurs with the intersite ranking of disturbance relative to recovery rate² or with the ranking of 'stress'¹, the independent variables in Horn's and Grime's theories respectively. A valid test requires that other important factors vary relatively little among sites, compared with D . This requirement is probably satisfied, considering the similar exposure and topography, soil depth, plant growth form, species composition and vegetative cover among sites.

Figure 1a, b shows that both SD and SR exhibit maxima at intermediate D , as predicted by theory. The heterogeneity among SD means is significant at $P < 0.001$, even after removing variance due to linear regression with D , indicating significant deviation from linearity. Requisite assumptions for the one-way analysis of variance, homogeneity of variances and normally distributed errors, were verified. A runs test also shows ($P < 0.002$) deviation about the linear regression of SD on D , in

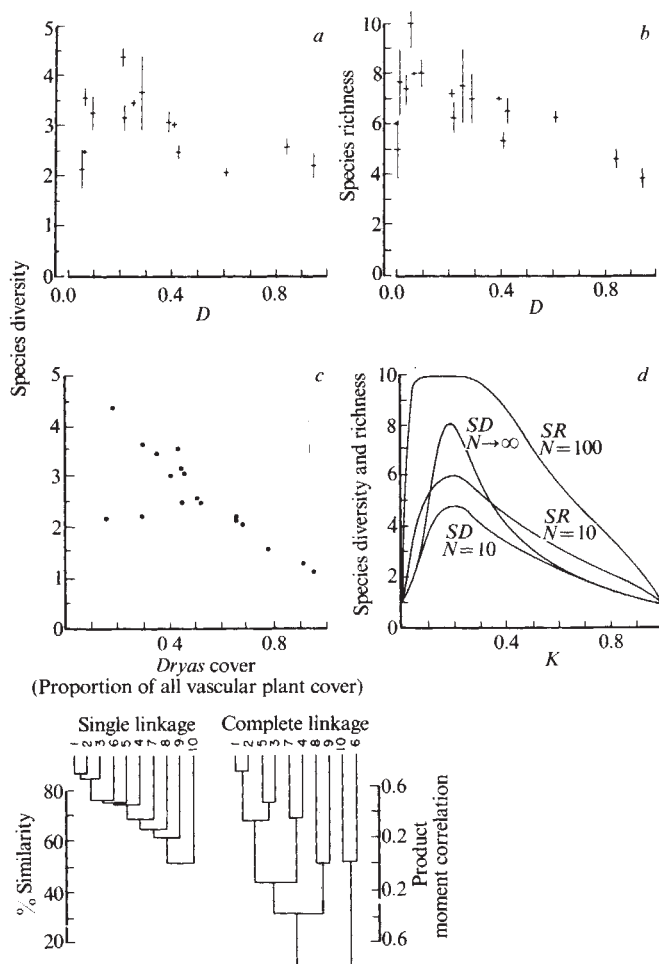


Fig. 1 a, b, Species diversity (SD) and richness (SR) versus soil frost disturbance (D). Horizontal bars = means; vertical bars = standard errors. c, Dependence of species diversity on cover of the dominant fellfield vascular species, *D. octopetala*. d, Theoretical sampled species diversity and richness for Horn's² relay-succession model. e, Dendrograms illustrating cluster analyses of the matrix of correlation coefficients between species pairs, among 18 sites; the similarity and correlation scales are equivalent. Species codes: 1, D (=the proportion of ground recently frost-disturbed); 2, *Antennaria monocephala*; 3, *Arenaria arctica*; 4, *Oxytropis nigrescens*; 5, *Diapensia lapponica*; 6, grasses (predominantly *Hierochloa alpina*); 7, *Salix phlebophylla*; 8, *Loiseluria procumbens*; 9, *Arctostaphylos alpina*; 10, *Dryas octopetala*.

the direction expected by hypothesis. For *SR*, the heterogeneity is not significant; this is most probably due to insufficient sample size. The index of *SD* used here is strongly influenced by evenness^{20,21}, and so evenness (*SD/SR*), not unexpectedly, follows the same relationship as *SD* to *D*. Second- and third-degree polynomials offer significant improvements in fit over a linear model for *SD* as a function of *D* or $\ln D$. The analysis therefore conclusively rejects null hypotheses that *SD* is related to *D* and $\ln D$ either nonsignificantly or by a purely linear model. *Dryas* cover is highly correlated with *SD* ($r = -0.774$, $P < 0.001$, Fig. 1c) but not with *SR* ($r = 0.038$), indicating that dominance by this species is singularly instrumental in reducing diversity, and conversely that breaking up of *Dryas* mats by frost action is crucial to increased evenness of cover by other species.

The parametric species richness may be the same for all the study sites; sampled *SR* will inevitably appear larger at intermediate-disturbance sites where greater evenness of relative abundances ensures that, on average, more species will occur in small samples. Parametric *SR* (such as S_T of May²⁰), the theoretical maximum number of species in a community, is usually underestimated by sampled *SR*. The effect of *D* on sampled *SR* is shown using Horn's² relay-succession model, with S_T fixed and *D* influencing only evenness. Sample estimates for both *SD* and *SR* were made numerically by sampling the stationary distribution²,

$$p_i^* = K(1-K)^{(i-1)}, \quad i = 1, 2, \dots, S-1, \quad p_S^* = (1-K)^{S-1}$$

For each value of *K*, random samples of $N = 10$ and $N = 100$ were taken 50 times and the 50 results averaged to obtain the sample estimate for that *K* and *N*. Here p_i^* represents the average relative abundance of species *i*; $S = S_T = 10$; and *K* is an arbitrary parameter, $0 < K < 1$, governing the evenness of species abundances (*K* is equivalent to Horn's² $d/(d+e)$). In Horn's model, *K* increases monotonically with disturbance, and *SD* (evenness) is maximized at intermediate *K*. Figure 1d shows that a unimodal curve is expected for *SR*, with a peak flattening out and moving left as S_T is reached in sufficiently large samples. Although *K* is nonlinearly related to *D*, there is a 1:1 relationship between the *K* and *D* axes in Fig. 1. This simulation demonstrates that the general shape of the sampled *SR-D* curve provides no evidence that S_T is influenced by *D*, although that is one claim of the disturbance hypothesis. Figure 1b therefore provides no evidence that the *SR* component of *SD* is affected by *D*. *D* may be affecting only the evenness component of *SD*, but more detailed study is required to confirm this. The theoretical problem of how disturbance influences S_T remains unexplored in any rigorous sense.

Finally, the fellfield data are consistent with a microsuccessional process. The matrix of species × species correlation coefficients (based on species relative abundances for the 18 sites) exhibits an ordered, non-random pattern best revealed by cluster analysis^{22,23}. In Fig. 1e, there is marked concordance between the two extreme clustering methods (single and complete linkage), and a pronounced serial hierarchy or chaining^{22,23} appears in the dendrograms. The single linkage chain is unbroken from highest to lowest linkage; the probability of such a chain, assuming any one of a species' $S-1$ correlations with other species may be its highest, is $(S-2)!/(S-1)^{S-3}$, in this case ($S = 10$), $P = 0.008$. A species' highest correlation is not necessarily with the one to its immediate left, suggesting that successional trajectories are more variable or complex than those of simple relay succession². The evident hierarchy in correlation among species would be expected in a collection of samples from a fairly simple successional mosaic, rather than from an assemblage of mature communities.

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Isozyme analysis indicates that a virulent cereal rust pathogen is a somatic hybrid

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Somatic hybridization between *formae speciales* or different strains of the same *forma specialis* of cereal rusts has often been implicated in the origin of new virulence combinations^{1–3}. It has been suggested^{4,5} that somatic hybridization between *Puccinia graminis* f.sp. *secalis* (rye stem rust) and *P. graminis* f.sp. *tritici* (wheat stem rust) has given rise to hybrids virulent on rough wheat grass, *Agropyron scabrum*, and on barley in Queensland, Australia. This hypothesis is supported by differences in the virulence characteristics of the hybrids and the putative parental strains. To date, verification of the origin of the hybrids (scabrum rusts⁴) has not been possible. Here we report that isozyme differences between f.sp. *secalis* and f.sp. *tritici* provide evidence that scabrum rusts are natural somatic hybrids.

Isolates of rye stem rust, wheat stem rust and their putative hybrids were collected in the same geographical region on *Secale cereale*, *Triticum aestivum* and *A. scabrum* (a mutual host for all three types) respectively. Putative hybrids were identified by their virulence characteristics on selected cultivars of rye and wheat⁵. Individual isolates were grown in isolation on suitable hosts and the isozyme banding pattern of several enzymes was studied by separating extracts of germinating urediniospores by horizontal starch gel electrophoresis⁶ and then specifically staining the gels for esterase (EST), glutamate dehydrogenase (GDH), glutamate oxalo-transaminase (GOT), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), NADH diaphorase (NADHD) and phosphoglucumutase (PGM). The buffer systems and staining procedures have been described elsewhere^{7–9}. Eleven, twenty and fourteen different isolates of f.sp. *secalis*, f.sp. *tritici* and their putative hybrids were examined respectively.

Of the enzymes investigated, no differences were detected between the isozyme banding patterns of GDH, GOT and MDH. Each was characterized by a single, invariant band. This result is consistent with previous evidence that f.sp. *secalis* and f.sp. *tritici* are closely related^{6,10}.

When taken together, the isozyme patterns for NADHD and LAP provide strong support for a hybrid origin of the scabrum isolates. For LAP, all putative hybrids have an isozyme phenotype identical to f.sp. *secalis*, while for NADHD all putative hybrids are identical to f.sp. *tritici* (Table 1).

Similarly, the results obtained with the PGM system support a somatic hybrid origin. 10 of the 11 f.sp. *secalis* isolates had a very slowly migrating band not found in the f.sp. *tritici* isolates (Table 1) and yet 10 of the 14 scabrum types possessed this band.

Table 1 Diagrammatic representation of the diagnostic isozyme banding patterns for *Puccinia graminis* f.sp. *tritici*, f.sp. *secalis* and their putative somatic hybrids

Isozyme system	Pathogen					
	<i>P. graminis tritici</i>	Putative hybrid			<i>P. graminis secalis</i>	
		or			or	
EST	—	—	—	—	—	—
LAP	(20)	(10)	(4)	(1)	(10)	
NADHD	(20)	(14)			(11)	
PGM	(20)	(14)			(11)	
		or	or	or	or	
	—	—	—	—	—	—
	(20)	(4)	(6)	(3)	(1)	(10)

Figures in parentheses are the numbers of each phenotype.

Moreover, all the wheat stem rust isolates possessed a band of intermediate mobility not present in any of the f.sp. *secalis* isolates, but present in 13 of the 14 putative hybrids. Finally, in the scabrum types, the enzyme banding patterns for EST and PGM are inversely correlated. All scabrum types possessing the f.sp. *secalis* three-banded EST pattern have the f.sp. *tritici* PGM pattern. On the other hand, all putative hybrids with the unique f.sp. *secalis* PGM slow band have the same EST pattern as f.sp. *tritici*.

The occurrence and constancy of the inverse correlation between the f.sp. *secalis* and f.sp. *tritici* EST and PGM banding patterns in the putative hybrids and the distribution of the LAP and NADHD patterns clearly confirm the postulated hybrid origin of the scabrum types^{4,5}. There are three possible explanations for the observed inverse correlations among the enzyme systems: (1) the enzyme variants themselves are under intense selection and determine the survival of pathogen biotypes on particular hosts; (2) the correlated enzyme variants are closely linked with each other and a third locus, such as an avirulence/virulence locus which is under intense selection; or (3) the hybrids represent the products of whole nuclear exchange which limits the number of potential recombinant types. The first of these possibilities opposes a substantial body of literature¹¹ which discounts the possibility of strong selective pressure on individual isozyme variants *per se*. Similarly, the second possibility can be discarded on the basis of our unpublished work which indicates, within a *forma specialis*, little correlation between isozyme variants and virulence characters. Thus considering the dikaryotic nature of these rusts, it would seem that whole nuclear exchange provides the most satisfactory explanation for the observed isozyme correlations. Evidence has previously been obtained^{2,3} for whole nuclear exchange in the formation of somatic hybrids in other *Puccinia* species.

Regardless of the mechanisms involved, the present study suggests that somatic hybridization occurs between different *formae speciales* of *Puccinia*. Isozyme marker loci clearly provide a simple and reliable way of detecting such hybrids. Application of this technique to different races of the same *forma specialis* should provide evidence concerning the relative frequency and importance of this mechanism for producing races with new combinations of virulence.

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Direct measurement of the force of microtubule sliding in flagella

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The movement of eukaryotic cilia and flagella is caused by ATP-driven active sliding between the doublet microtubules¹⁻³, and the force for the sliding is believed to be generated by the dynein arms of the A-tubule interacting with the B-tubule of the adjoining doublet. To understand the mechanochemical basis of this force-generating reaction and to correlate it with the overt motile behaviour of cilia or flagella, it is important to quantify the force exerted by the dynein arms. Attempts have been made to estimate this force from the bending moment generated by the whole organelle^{4,5}, but the estimation was of necessity hypothetical because the mechanism by which sliding is coupled with bending is poorly understood. Microtubule sliding without bending can be induced *in vitro* if trypsin-² or elastase⁶-treated axonemes are perfused with a solution containing ATP. By attaching glass microneedles to such axonemes, we have now directly determined the sliding force at various ATP concentrations.

Sea-urchin (*Hemicentrotus pulcherrimus*) sperm was demembranated with Triton X-100⁷. A single sperm was then attached at two sites to a pair of polylysine-coated glass microneedles³ mounted on micromanipulators (Figs 1a, 2a). One of the needles was relatively stiff, with a sharply tapered tip and was used to hold one end of the axoneme. The other needle was more flexible and was used to measure the sliding force. The compliance of the needle had been determined by a cross-calibration with a needle of a known compliance⁸. Usually, needles with an elastic coefficient of ~ 100 pN μm^{-1} were selected for the experiment.

When the demembranated sperm was perfused with a solution containing ATP and elastase, it was at first reactivated to beat. After about 1 min, however, the beating stopped and the axoneme subsequently underwent a process of sliding disintegration. In many cases, as the microtubules slid over each other, the flexible needle was pulled towards the stiff needle (Figs 1, 2). The movement was not always smooth: often the needle stopped temporarily, or moved back and then moved forwards again. It is possible that these distinct pulls represent sliding between different pairs of doublets. However, in a few cases the flexible needle moved back and forth more than nine times, indicating that in our experimental conditions, sliding can occur more than once between a pair of doublets. The movement of the needle was recorded with a 16-mm cine camera, and the force was measured from the film as the elastic displacement

Table 1 Maximal sliding force developed in various concentrations of ATP

ATP concentration (μM)	Force $\pm$ s.d. ($\times 10^{-11}$ N μm^{-1})	n	Range ($\times 10^{-11}$ N μm^{-1})
4	2.0 ± 0.7	21	0.85–4.1
20	2.8 ± 1.8	22	0.56–8.8
200	3.5 ± 1.8	28	1.1–8.9
500	5.7 ± 1.7	5	3.8–8.3

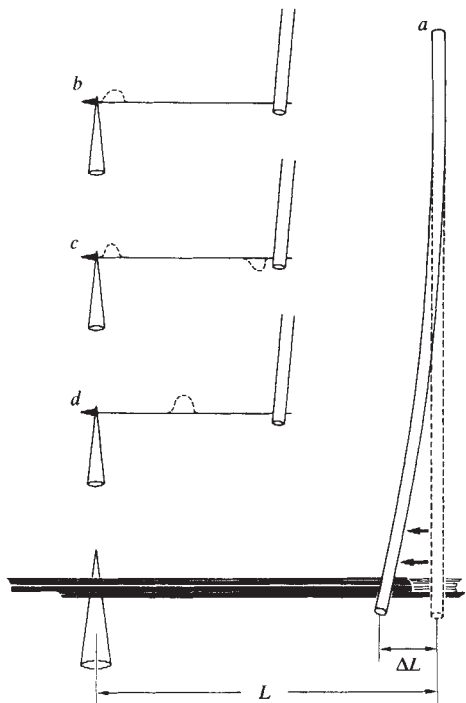


Fig. 1 Diagrams illustrating the principle of the experiment. Spermatozoa of the sea urchin, *Hemicentrotus pulcherrimus*, were demembrated with the extracting solution (0.15 M KCl, 4.0 mM MgSO₄, 0.5 mM EDTA, 2.5 mM CaCl₂, 0.04% Triton X-100, 1.0 mM dithiothreitol, 10 mM Tris, pH 8.0) and stored for up to 1 h in the reactivating solution without ATP (0.15 M KCl, 2.0 mM MgSO₄, 2.0 mM glycoethyldiamine-*N,N,N',N'*-tetraacetic acid, 1.0 mM dithiothreitol, 10 mM Tris, pH 8.0) at 0 °C. Experiments were performed at 20 ± 1 °C. *a*, The demembrated sperm was attached at two sites to a pair of microneedles—a stiff needle (left) and a flexible needle (right). It was perfused first with the reactivating solution containing ATP and then with the solution containing both ATP and elastase (10 μg ml⁻¹). The latter solution induced sliding disintegration of the axoneme within about 1 min. In many cases the flexible needle was pulled towards the stiff needle as a result of microtubule sliding in the portion of the axoneme between the needles. Δ*L* is the displacement of the flexible needle, *L* is the initial distance between the two needles. *b*, *c*, *d*, Frequently, microtubules were observed to loop out from the axoneme.

of the needle; the maximal force is the force measured when the needle stopped in a fully pulled position.

The maximal force generated by a single dynein arm was calculated by the equation $f = k\Delta L/n(L - \Delta L)$, where *k* is the elastic coefficient of the flexible needle, *n* the number of dynein arms per unit length of the doublet (here assumed to be 83 per μm)⁹, *L* the initial distance between the two glass microneedles and Δ*L* the maximal displacement of the flexible needle towards the stiff needle (Fig. 1*a*). The equation assumes that the force is generated along the whole length of one doublet between the microneedles—admittedly a rough assumption. In some cases microtubules were observed to loop out from the axoneme (Fig. 1*b–d*); *f* may then be underestimated because the true length of the force-generating region may be less than *L* - Δ*L*. In contrast, *f* will be overestimated if the sliding force is generated effectively in more than one interdoublet sites at the same time. Close observation of the sliding process indicated, however, that the latter case, which requires precisely synchronized sliding at two or more interdoublet sites, was rather unlikely.

The maximal force developed by a unit length of doublet microtubules was determined in four different ATP concentrations (see Table 1). As our procedure tends to underestimate the force, the largest value obtained in each ATP concentration seems to be closest to the true value. In this respect, it is interesting that the largest values are about the same in ATP concentrations between 20 and 500 μM. By using these values,

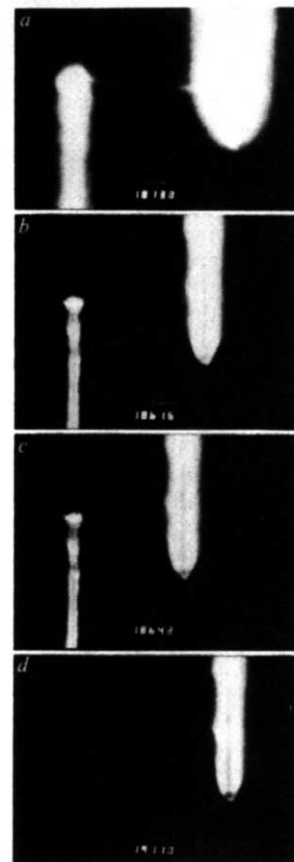


Fig. 2 Prints from a 16-mm cine film, showing a movement of the flexible needle attached to an axoneme as in Fig. 1. The flexible needle (right) had an elastic coefficient, *k*, of 1.9×10^{-10} N μm⁻¹. *a*, Before the onset of movement. Photographed with increased illumination to show, between the needles, the axoneme which cannot be seen with the light intensity suitable for the needles. *b*, *c*, The flexible needle is pulled towards the stiff needle. *d*, The stiff needle with the attached axoneme was removed to show the position of flexible needle at zero force. The number at the bottom of each frame is a clock giving time in 0.1 s. Scale bar, 20 μm.

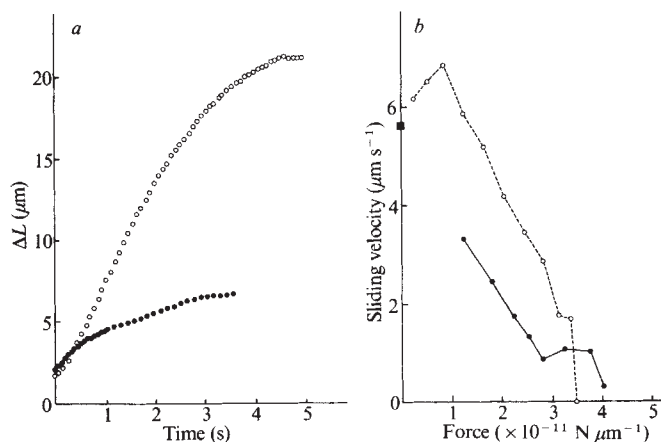


Fig. 3 *a*, Displacements (Δ*L*) of the flexible needle in two experiments are plotted against time. ●, Result obtained with a needle which had an elastic coefficient, *k*, of 1.8×10^{-10} N μm⁻¹. The initial length of the axoneme between the needles (*L*) was 38 μm. ○, Result with a more flexible needle ($k = 2.7 \times 10^{-11}$ N μm⁻¹, *L* = 36 μm). ATP concentration, 200 μM. *b*, Relationship between the sliding velocity and the load given to a unit length of the microtubule. Velocities were calculated by the least-squares method from every five dots in *a*, and replotted against load ($= k\Delta L/(L - \Delta L)$). Symbols are as in *a*. The velocity of unloaded microtubule sliding in trypsin-treated axonemes at 200 μM ATP (unpublished) is shown by ■.

the maximal force per dynein arm is tentatively calculated to be about 1 pN.

In our experiments, the load on the sliding microtubules slowly increases with the bending displacement of the flexible needle (Fig. 1a). By analysing the movement of the needle, therefore, the relationship between the load and the velocity of microtubule sliding can be determined. Figure 3a shows two examples of needle displacement (ΔL) plotted against time. From this, the sliding velocities were calculated by applying the least-squares method to every five successive points. The results are replotted against force per unit length of the microtubule in Fig. 3b, which shows that the sliding velocity decreases as the load increases.

This is the first direct measurement of the sliding force in the dynein-tubulin system. Although our assumptions contain some uncertainties, it is interesting that the value we obtained is of the same order of magnitude as previous estimates of the maximal force (2–9 pN per dynein arm) made on less direct bases^{4,5}. It is also interesting that similar values (2–5 pN) have been obtained as an estimate of the sliding force generated by a myosin cross-bridge in the striated muscle^{10,11}.

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Primitive mathematical concepts in the chimpanzee: proportionality and numerosity

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Comparison of quantitative reasoning in nonhuman animals has suffered, on the one hand, from the methodological failure to do properly controlled studies of number (for review, recent examples and exceptions see refs 1, 2; 3, 4; and 5, 6 respectively), and on the other, from the conceptual failure to consider forms of quantitative reasoning other than number. An approach to mathematical reasoning may profit from the study of proportion, a continuous quantity, in addition to number, a discrete quantity. In the experiments reported here, an adult chimpanzee and four juveniles were tested for their knowledge of 'proportion' and 'number' with conceptual match-to-sample tasks. The juveniles failed but the adult successfully matched exemplars of the proportions 1/4, 1/2, 3/4 and 1, and the numbers 1, 2, 3 and 4, when the sample and alternatives were highly dissimilar physically (such as in shape, colour) and in other quantitative (for example mass, area, length) dimensions. The results reveal the presence of simple 'proportion' and 'number' concepts in a nonhuman primate.

The subjects were Sarah, an adult female chimpanzee, and four juveniles, three females and a male (*Pan troglodytes*), all African born and received in the laboratory as infants. All five animals had been trained previously in numerous tasks, but only Sarah had been trained in a simplified language⁷. As the non-language-trained animals could not be tested with

same/different judgements, we used match-to-sample, a paradigm on which all the animals were highly trained. In each trial, the subject was required to choose one stimulus, from a set of two, that was identical to the sample on a specified dimension. The stimuli were drawn from three classes of object: food items roughly spherical in shape (apple, grapefruit, potato; diameters 5–15 cm), circular wood disks painted natural grey (2 cm thick, with diameters of 2.5–17.5 cm) and cylindrical columns of water tinted blue with food colouring (in Plexiglas jars, 8 cm in diameter and 20 cm high, or metal measuring cups, 8.5 cm in diameter and 5 cm high).

The same stimuli were used in both the proportions (1/4, 1/2, 3/4, 1) and numbers (1, 2, 3, 4) tests. One or the whole object in the proportions test and '1' in the number test were both represented by a single intact food item, disk, or container (jar for proportions, cup for numbers) filled to the brim with water. Exemplars of '1/4', '1/2' and '3/4' were prepared by removing sections from foods or disks by vertical cuts, or in the case of liquids, by filling Plexiglas jars to the appropriate levels with water. Exemplars of '2', '3' and '4' were constructed by arranging sets of food or disks at random in 25-cm diameter metal bowls, or, in the case of liquids, by affixing metal cups of water to 12 × 25 cm wood strips, for ease of handling by the subject. Figure 1 shows a photograph of the kinds of exemplar used in the experiment.

In the first block of tests, all three stimuli per trial (one sample and two alternatives) were taken from the same class of object, either sections or sets of the same type of food item (phase 1), container of liquid (phase 2), or wood disk (phase 3). These trials were intended merely to familiarize the subjects with the stimuli and to ensure that they could distinguish between all the stimuli in each class of object.

The critical testing began in the second block of tests, where the sample was always a liquid exemplar, whereas both alternatives were either food items of the same type (phase 4) or wood disks (phase 5). In addition, the correct and incorrect alternatives were always equated for size (mass in the case of foods, surface area in the case of disks). Thus, the correct alternative could not be differentiated from the incorrect one on the basis of physical resemblance to the sample (size or amount, shape, colour and so on). Successful performance in these trials required accurate matching of the two exemplars differing in physical detail, but alike in proportionality or numerosity.

Within each phase, sequences of trials with the proportional or numerical exemplars were presented in an ABBA design (18 to 24 trials with exemplars of proportion, 36 or 48 trials with exemplars of numbers, and finally, 18 or 24 trials with exemplars of proportions). Exemplars of each of the four proportions and four numbers were presented as samples equally often in counterbalanced fashion. Left-right position of the correct alternative and type of food or size of wood disk was also counterbalanced across trials. On trials with a proportional sample, the incorrect alternative was selected from the set of three remaining proportions such that all exemplars appeared equally often during testing. Similarly, in trials with a numerical sample, the incorrect alternative was one of the set of three remaining numbers, each appearing equally often.

Table 1 Number of trials with a correct choice/total trials on the most difficult discriminations, between adjacent quantities, taken from within-class matching trials in phases 1, 2 and 3 (top) and from between-class matching trials in phases 4 and 5 (bottom). Data are for Sarah

	Adjacent quantities					
	Proportions			Numbers		
	1/4:1/2	1/2:3/4	3/4:1	1:2	2:3	3:4
Trials from first test block	16/20	18/20	16/20	18/20	20/20	17/20
Trials from second test block	13/14	12/14	12/14	12/14	12/14	13/14

All $P < 0.05$; binominal test.

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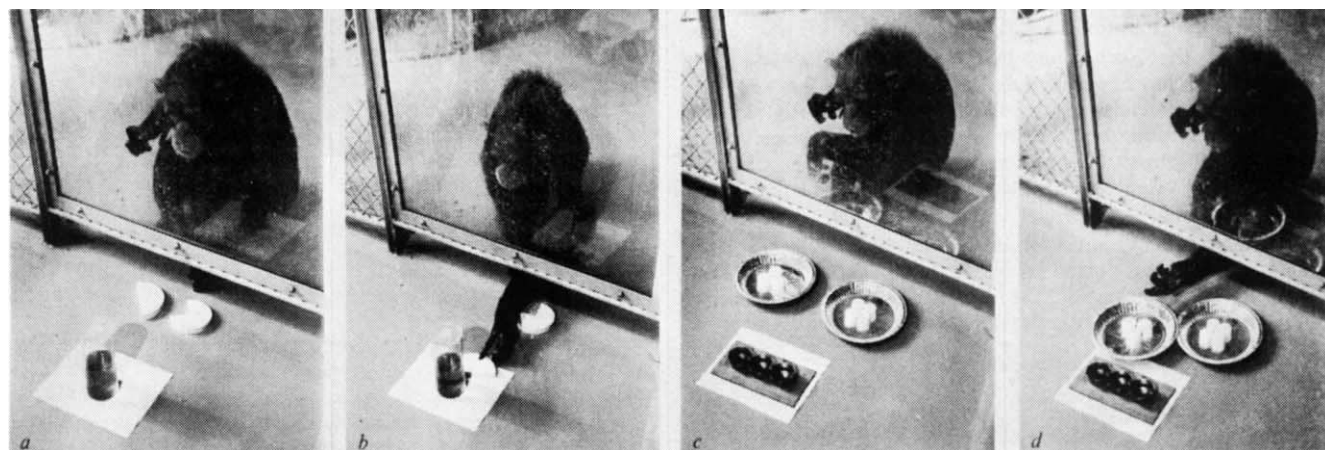


Fig. 1 Physical arrangement of test stimuli during matching trials in phase 5 of the second block of tests. In *a*, Sarah is confronted with a liquid exemplar of '1/2' on the white paper (the sample) and two wooden-disk alternatives, exemplifying '1/2' and '3/4'. In *b*, she chooses the '1/2' disk and places it with the sample on the paper. In *c*, Sarah examines a liquid exemplar of '3' on the paper, and two sets of wooden disks presented in metal pans, exemplifying '3' and '4'. In *d*, she chooses the pan containing three disks and places it with the sample on the paper. (Both alternatives were presented in a cardboard box during the experiment proper. The photographs shown here were taken after the conclusion of the experiment and for illustrative purposes the box was removed.)

Sarah was tested in her home cage with a procedure that controlled for inadvertent social cues⁸. A standard control for social cues was used with the juveniles. An opaque barrier separated the two trainers such that the trainer presenting the sample could not see the alternatives, and conversely, the trainer presenting the alternatives could not see the sample; only the subject could see both the sample and alternatives. Sessions consisted of 12 or 18 trials, separated by inter-trial intervals of ~1 min, and were conducted five times per week.

In the first block, Sarah responded correctly in a significant proportion of all trials with proportional or numerical exemplars from each class of object. Her overall matching scores (number of correct/total trials) with proportional exemplars were 35/36, 32/36 and 39/48 in phases 1, 2 and 3 respectively. Her corresponding scores with numerical exemplars were 31/36, 32/36 and 48/48 (all $P < 0.001$, binominal test). The data in Table 1 show her performance in the most difficult discriminations, those between exemplars of adjacent quantities (1/4 versus 1/2, 1/2 versus 3/4, 3/4 versus 1; 1 versus 2, 2 versus 3, 3 versus 4). Sarah responded at levels significantly above chance to all adjacent pairs, equally for proportions and numbers.

The juveniles responded in a similar manner, for example, except for one animal in phase 1, they responded correctly in a significant proportion of all trials with proportional or numerical exemplars from each class of object. However, they were less accurate than Sarah and their inaccuracy was especially apparent in the adjacent discriminations. They all discriminated 3/4 from 1, but only two of them discriminated 1/4 from 1/2 and none discriminated 1/2 from 3/4. They performed even worse with adjacent number pairs. For example, in some subjects, 'runs' of consecutive correct responses for judgements of the number either failed or took longer to appear. Pairwise comparisons of the number of trials to the beginning of a run in proportionality with that in numerosity trials showed a significant difference (paired t -test = 3.29, d.f. = 14, $P < 0.01$).

Although Sarah and the juveniles responded similarly in the first block of tests (where the sample and correct alternative could be matched in terms of physical appearance), they did not respond in the same way in the second test block (where the sample and correct alternative could not be matched in terms of physical appearance). The juveniles failed all phases of the second test block, whereas Sarah continued to respond at levels significantly above chance. Her overall matching scores with proportional exemplars were 26/36 and 42/48 in phases 4 and 5 respectively, and with numerosity exemplars, 30/36 and 41/48 (all $P < 0.01$, binominal test). Indeed, she responded at levels exceeding chance from the very first trial of each phase, showing a significant 'run' of consecutive correct responses beginning in

trial 1 (phase 4; proportions, 10 of the first 10 trials correct; all $P < 0.01$, Grant's runs test). Lastly, the data presented in Table 1 indicate that in the adjacent-pair discriminations, Sarah performed at the same high level in the second block of tests as she had in the first block, and did so both for proportionality and number.

Is it possible that Sarah's strong performance was based not on the concepts of number and proportion, but simply on having learned to associate particular samples and alternatives? To assess this possibility, we examined her choices in the first trial with each of the four proportions and four numbers in phases 4 and 5 (total of 16 trials). Sarah chose correctly in 8/8 proportionality trials and 8/8 numerosity trials (both $P < 0.01$; binominal test). These first-trial data rule out the possibility that she learned simply to choose the correct alternative for each type of sample and, together with the fact that she showed a significant run of consecutive correct responses beginning with the first-trial in each between-class test, strongly support the conclusion that Sarah matched stimuli on the basis of proportionality and numerosity in the critical second test block.

What mechanisms does the chimpanzee use in making quantitative judgements, and are they like those used by humans? Although judgements of 'small' numerosities like those used here are ordinarily attributed to subitizing⁹—'immediate perceptual apprehension' of numerosity—children are known to count at an earlier age than previously supposed. They often count with a series that is idiosyncratic, such as 2, 6, 4, but which nonetheless is ordered, set into 1:1 correspondence with the items counted and used with the cardinality rule¹⁰. That is, the young child's use of, for example, '2, 6, 4', may be as correct as the older child's use of '1, 2, 3'. There is no comparable evidence for the ape, and although the ape may yet be taught to count, this has not been achieved so far.

One may subitize or see at a glance that two small numerosities are the same, but how does one see at a glance that, for example, 3/4 apple is the same as 3/4 column of water? It has not been established how proportionalities like those used here are judged by humans; a minimum requirement is that one be able to recognize the relationship between the part and whole in each of the cases compared. Then, one can proceed in one of the two directions: (1) label corresponding part-whole relationships the same, for example, call 1/4 apple and 1/4 column of liquid 'quarter', or (2) use a process of reasoning akin to analogy. For example, to match 1/4 apple to 1/4 column of liquid, one must appreciate that the relationship between the observed apple section (x) and the whole apple (X) from which it came is the same as that between the partially filled jar (y) and the completely filled jar (Y) from which it was derived (that is, x is to

X as y is to Y). The analogy may be considered more difficult because neither X (whole apple) nor Y (filled jar) is physically present and, therefore, must be inferred. Nevertheless, the possibility that Sarah reasoned in this manner here is in keeping not only with previous results showing her inferential ability with other quantitative properties¹¹, but also more recent data from our laboratory showing explicitly that she is capable of analogical reasoning¹². It seems unlikely that she could have taken the alternative approach, a common label, because she had not been taught names for any of the proportions.

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Specific stimulation of *in vitro* maturation of mesencephalic dopaminergic neurones by striatal membranes

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We have previously established that dissociated mesencephalic dopaminergic (DA) neurones from embryonic mouse differentiate in primary cultures and that their development is enhanced when they are grown in the presence of their target cells from the striatum¹⁻³. The capacity of individual DA neurones to take up and synthesize ³H-DA was increased in co-culture. This phenomenon does not depend on the presence of glial cells because it also occurred in serum-free conditions in which glial development was largely impaired⁴. Therefore, striatal target neurones favour the maturation of afferent DA neurones. Diffusible or membrane-bound factors could be involved in this process. To test one of these possibilities, we have now examined the *in vitro* development of embryonic mesencephalic DA neurones exposed to striatal membranes isolated from postnatal mice at various ages. ³H-DA uptake was used as an index of maturation of the DA neurones⁵. We show that striatal membranes from 2- and 3-week-old animals stimulate the development of DA neurones. The finding that this effect was not seen with membranes from 1-week-old or adult animals suggests that a developmentally regulated membrane-bound 'factor' is probably involved. This factor seems to be specific for the DA neurones.

When dissociated mesencephalic cells from 13-day-old mouse embryos were cultured for 3 days in the presence of crude membranes from 2- or 3-week-old mouse striatum, ³H-DA uptake capacity of the DA neurones was strongly stimulated (Fig. 1). Two control experiments confirmed that this effect, which increased with the concentration of membrane used, cannot be attributed to a specific or nonspecific binding of ³H-DA to added membranes. First, when striatal membranes from 3-week-old animals were mixed with striatal cells from

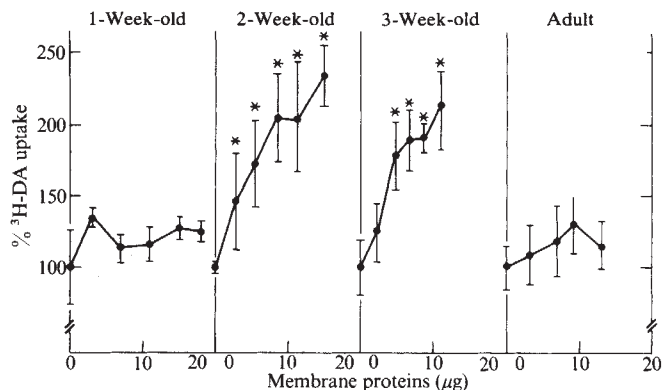


Fig. 1 Effects of striatal membranes prepared at various ages on ³H-DA uptake in mesencephalic DA neurones. Dissociated embryonic mesencephalic cells from 13-day-old Swiss mouse embryos were prepared as previously described⁴ with the following modifications. Polyornithine (1.5 μg ml⁻¹, Sigma) coated multiwell tissue culture plates (Falcon) were used and antibiotic concentration in the culture medium was raised (5 IU and 5 μg ml⁻¹, respectively, for penicillin G and streptomycin). Membranes were prepared by gentle homogenization of fresh brain tissue (~10 mg) with a Kontess potter. The homogenization buffer (1 ml) was composed of 5 × 10⁻² M Tris-HCl pH 7.4, 32 × 10⁻² M sucrose, 10⁻³ M magnesium, 10⁻³ M calcium, 10⁻² M β-mercaptoethanol and 10% fetal calf serum. Homogenized membranes were spun at 30,000g for 1 h at 4 °C. The pellet was resuspended in 1 ml of serum containing culture medium and recentrifuged for 1 h at 30,000g and 4 °C. The supernatant was discarded and the pellet resuspended in the same medium was used as the source of crude membranes. No stimulatory activity was found in either supernatant. Mesencephalic cells (600,000 per well) were mixed with various quantities of membranes (0-25 μl) and plated in serum-complemented medium. The total volume of medium per well was adjusted to 0.3 ml. The protein content of crude membrane preparations was measured according to Lowry¹⁴. ³H-DA uptake was estimated after 3 days in culture. Cells were incubated for 5 min at 37 °C in 0.25 ml phosphate buffer containing 3.3 × 10⁻² M glucose, 10⁻³ M magnesium and 10⁻³ M calcium (complete PBS) in the presence of 5 × 10⁻⁸ M ³H-DA (15 Ci mmol⁻¹, Amersham). The cells were then rinsed three times with cold complete PBS and the quantity of radioactivity accumulated in tissues was determined as previously described¹. Results are expressed as per cent of control values (without membrane). Each value is the mean ± s.e.m. of data obtained with four cultures. * $P \leq 0.05$ when compared with respective control values (Student's *t*-test)

15-day-old embryos and ³H-DA uptake was estimated after 3 days in culture, ³H-DA uptake or binding was negligible in the absence of mesencephalic DA neurones (in c.p.m.: 59 ± 2, 59 ± 3 and 69 ± 6 for 0, 13 and 26 μg of membrane protein, respectively). Second, as expected, total ³H-DA accumulation in cultures of mesencephalic cells with striatal membranes was reduced to background level (60 c.p.m.) in the presence of benztrapine (5 × 10⁻⁷ M), a specific inhibitor of DA uptake in DA neurones, compared with a ³H-DA uptake value of 280 ± 30 c.p.m. in the absence of benztrapine. The benztrapine-induced reduction in ³H-DA uptake occurred regardless of the concentration of membranes used (in c.p.m.: 83 ± 12, 74 ± 10, 82 ± 4 and 99 ± 8 for 0, 10, 20 and 25 μg of membranes, respectively). We therefore conclude that the stimulatory effect of striatal membranes from 2- and 3-week-old mice on ³H-DA accumulation is related to an enhanced ³H-DA uptake capacity of mesencephalic cultures. This effect on ³H-DA uptake disappeared when the striatal membranes were denatured at 90 °C for 10 min or prepared in the absence of fetal calf serum which is known to inhibit proteolytic activity. These observations suggest that the membrane-bound factor implicated is partially proteic in nature. Interestingly, this factor seems to be developmentally regulated because, in contrast to membranes from 2- and 3-week-old mice, striatal membranes from 1-week-old or adult animals did not stimulate the ³H-DA uptake capacity of mesencephalic DA neurones.

To demonstrate that striatal membranes from 2- and 3-week-old animals exert a specific stimulatory effect on the maturation or survival of DA neurones and not a general action on the overall neuronal population, we analysed the responsiveness to the striatal membranes of neurones containing γ-aminobutyric acid (GABA), which are also present in our mesencephalic cultures. For this purpose, ¹⁴C-GABA and ³H-DA uptakes

were simultaneously estimated in mesencephalic cells which had been cultured for 3 days with various concentrations of striatal membranes from 3-week-old animals. Incubations were performed in the presence of β -alanine (5×10^{-4} M), which prevents 14 C-GABA uptake into glial cells (the total uptake was reduced by 40%), and it was verified in one experiment that remaining 14 C-GABA uptake (171 ± 22 c.p.m.) was completely abolished (18 ± 2 c.p.m., $n = 4$) in the presence of L-2,4-diaminobutyric acid (10^{-3} M), a specific inhibitor of GABA neuronal uptake⁶. As described above, striatal membranes enhanced the 3 H-DA uptake capacity of mesencephalic DA neurones but, at all concentrations of striatal membranes used, had no significant effect on the capacity of GABA neurones to take up 14 C-GABA (Fig. 2). Results obtained in 14 experiments are shown in Fig. 3. For comparison, the stimulatory effect per 10 μ g of membrane protein was expressed as per cent of control values (without membranes) for 3 H-DA and 14 C-GABA uptakes. Although 3 H-DA and 14 C-GABA uptake capacities of mesencephalic cells varied between experiments, the stimulatory effect of striatal membranes was always much greater on 3 H-DA than on 14 C-GABA uptake. This is well illustrated by the increase in the 3 H-DA/ 14 C-GABA ratio calculated in each experiment (expressed as per cent of the control value obtained in the absence of striatal membranes). These data indicate that the DA and GABA mesencephalic neurones respond differently to the presence of striatal membranes. It remains to be established whether this is also the case for aminergic neurones other than DA neurones.

To test further the specificity of striatal membranes in stimulating the development of mesencephalic DA neurones, we analysed the effects of membranes prepared from other brain structures poorly or non-innervated by DA neurones. Parallel experiments were performed with various concentrations of

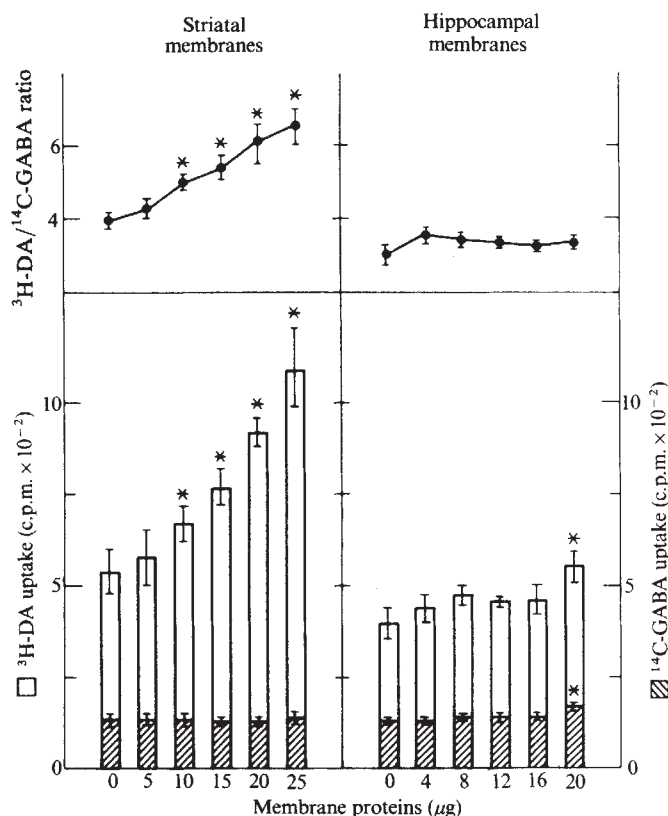


Fig. 2 Differential effect of striatal and hippocampal membranes on 3 H-DA and 14 C-GABA uptake. 3 H-DA and 14 C-GABA uptake studies in 3-day-old mesencephalic cultures were performed on the same cultures. Cells were incubated and treated as for 3 H-DA uptake (Fig. 1), but the incubation mixture was completed with 10^{-7} M 14 C-GABA (226 mCi mmol⁻¹, Amersham) and 5×10^{-4} M β -alanine. 3 H-DA/ 14 C-GABA uptake ratio was calculated in each culture done either with striatal or hippocampal membranes. Each value is the mean $\pm$ s.e.m. of data obtained with four cultures. * $P \leq 0.05$ when compared with respective control values.

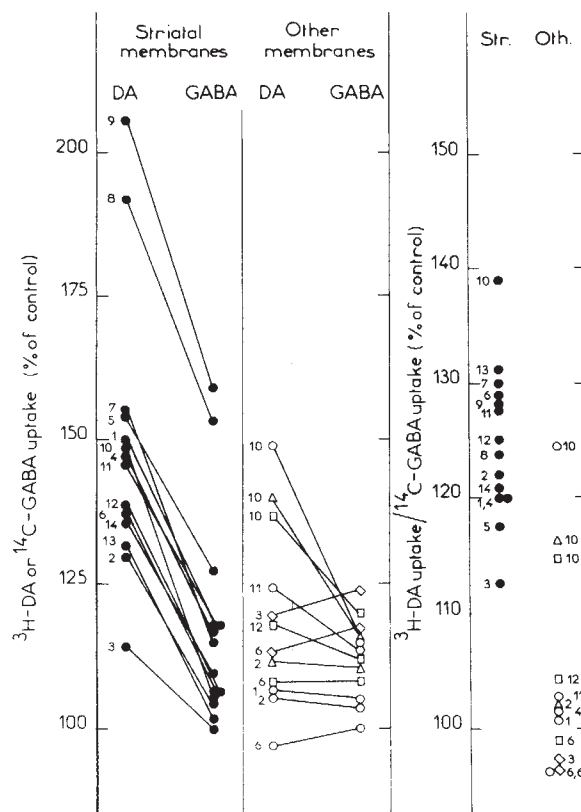


Fig. 3 Comparison of the effects of membranes from the striatum and from other brain structures on 3 H-DA and 14 C-GABA uptake in mesencephalic cells. 3 H-DA and 14 C-GABA uptake was estimated simultaneously as indicated in Figs 1 and 2. Cells were grown in the presence of striatal (●), hippocampal (□), cortical (parietal) (△), mesencephalic (◇) or cerebellar (○) membranes. The effects of 10 μ g of membrane proteins on 3 H-DA and 14 C-GABA uptake were expressed in per cent of respective control values (without membranes). Results obtained in each experiment (1–14) are illustrated by a line joining respective 3 H-DA and 14 C-GABA values. The numbers allow us to compare experiments made on the same day with the same preparation of mesencephalic cells but cultured in the presence of different types of membranes. The values of 3 H-DA/ 14 C-GABA uptake ratio calculated in each experiment for cultures made with striatal (●) or other types (○, □, △, ◇) of membranes are represented in the right panel. In each case, results are expressed in per cent of respective control values.

membranes from the hippocampus, which is practically devoid of DA innervation, and striatum of 2- or 3-week-old animals, measuring 3 H-DA and 14 C-GABA uptake capacities of mesencephalic cells and the 3 H-DA/ 14 C-GABA ratio in each culture. In contrast to results obtained with striatal membranes, no increase in 3 H-DA uptake was observed with most concentrations of hippocampal membranes used (Fig. 2). The slight but significant increase in 3 H-DA uptake observed with the highest membrane concentration (22 μ g protein) was associated with a parallel effect on 14 C-GABA uptake. Thus, whatever the concentration of hippocampal membranes used, the 3 H-DA/ 14 C-GABA ratio was never affected. This is very different from the situation with striatal membranes. A similar lack of effect was obtained with hippocampal membranes prepared from newborn, 1–3-week-old or adult animals (data not shown).

We also tested the effect of membranes from other brain structures, devoid of DA nerve terminals, from 2- or 3-week-old animals. Several experiments were carried out in parallel using striatal membranes as a reference (Fig. 3). In most cases, as observed for hippocampal membranes, cerebellar, parietal cortical or mesencephalic membranes did not affect differently 3 H-DA and 14 C-GABA uptake capacities of mesencephalic cells. The 3 H-DA/ 14 C-GABA ratio calculated in each case did not differ significantly from respective control values (obtained without membranes). In one experiment only (expt 10), although a high 3 H-DA/ 14 C-GABA ratio was seen with hippocampal, cerebellar and cortical membranes, striatal membranes were still more efficient in stimulating the maturation of the DA

Table 1 Comparisons of the effects of various striatal membrane concentrations on ^3H -DA uptake and the number of DA neurones in mesencephalic cultures

μg striatal membrane proteins	^3H -DA uptake (c.p.m.)	DA cell no.	^3H -DA uptake (c.p.m. per DA cell)
0	409 $\pm$ 71	954 $\pm$ 154	0.428
4	543 $\pm$ 39	1,083 $\pm$ 116	0.501
8	579 $\pm$ 116	1,116 $\pm$ 57	0.519
12	701 $\pm$ 58*	1,099 $\pm$ 121	0.637
16	734 $\pm$ 105*	1,207 $\pm$ 141	0.608
20	800 $\pm$ 28*	1,146 $\pm$ 58	0.698

^3H -DA uptake determination was performed as described in Fig. 1 legend. For autoradiographic studies, cells were incubated for 1 h at 37 °C in the presence of 5×10^{-7} M ^3H -DA and treated as described previously⁴. Each value is the mean $\pm$ s.e.m. of data obtained with four cultures.

* $P \leq 0.05$ when compared with respective control values.

neurones. A χ^2 distribution test performed on ^3H -DA/ ^{14}C -GABA ratios obtained with striatal membranes and pooled other membranes (including expt 10) showed the significance of the bipartition to be very high ($P \leq 0.001$). Therefore, striatal membranes from 2- or 3-week-old mice induce a specific stimulatory effect on the maturation of DA neurones, not shared by membranes originating from other structures containing few or no DA nerve terminals.

The specific stimulatory effect of striatal membranes on ^3H -DA uptake in mesencephalic cultures could reflect either an increased number of DA neurones or an enhanced capacity of each DA cell to take up ^3H -DA. Therefore, sister cultures of embryonic mesencephalic cells grown for 3 days in the absence or presence of increasing concentrations of striatal membranes from 3-week-old mice were used to estimate in parallel ^3H -DA uptake capacity and the number of DA neurones, by biochemical and autoradiographic analysis, respectively. As shown before, striatal membranes stimulated ^3H -DA capacity but the total number of DA cells in the cultures was not significantly modified whatever the concentration of membranes used (Table 1). A complementary experiment revealed that this enhanced capacity was due to a higher number of DA uptake sites per cell and not to a change in the affinity of DA for the membrane carrier (with and without membrane, respectively: V_{max} , 0.25 and 0.16 pmol min⁻¹; K_m , 2.2×10^{-7} M and 1.9×10^{-7} M). These results may reflect an increase in the density of uptake sites. However, as the number of uptake sites is generally used as an index of the extent of innervation, it can be proposed, as we did in our previous studies⁴, that the development of neurites per individual DA cell is enhanced in the presence of striatal membranes.

We conclude that striatal membranes from 2- and 3-week-old mice bear a factor(s) exhibiting protein properties which favour the early increased growth of mesencephalic embryonic DA cells in primary culture. Several findings suggest that the stimulatory effect of striatal membranes on DA neurone development is specific. (1) It is not observed with striatal membranes from 1-week-old or adult animals and therefore seems to be developmentally regulated, as described for the parasympathetic cholinergic factor⁷. (2) It does not occur with embryonic mesencephalic GABAergic neurones. (3) Hippocampal, cerebellar, cortical (parietal) and mesencephalic membranes are without significant action on the maturation of mesencephalic DA neurones. These latter findings are reminiscent of morphological observations made on aggregate co-cultures which revealed a preferential orientation of mesencephalic DA fibres towards cells of the striatum over those of the occipital cortex⁸.

It remains to be established whether the striatal membrane-bound factor revealed here is also responsible for the stimulatory effect of 15-day embryonic striatal cells on DA neurone development demonstrated in our previous co-culture experiments^{1,4}. This may not be the case because striatal membranes from 1-week-old mice did not enhance the maturation of DA cells. However, it can be speculated that direct contacts between DA and striatal neurones could induce an earlier expression *in*

vitro of the membrane-bound factor at the surface of embryonic striatal cells. Indeed, *in vivo*, the stimulatory effect of striatal membranes only appears during the second and third week of postnatal life, when contacts between DA nerve terminals and striatal cells are greatest. If this latter hypothesis is correct, it can be inferred that the membrane-bound factor originates from neurones and not from glial cells, because the stimulatory effect of striatal cells on mesencephalic DA neurones has also been observed in culture conditions impairing the development of glial cells (glial cells reduced by >95%).

As suggested by experiments revealing the specific recognition of the tectum by growth cones of retinal ganglion cells^{9,10}, the striatal membrane-bound DA factor could provide specific recognition sites favouring the ingrowth of the DA fibres in the striatum. Because, in contrast to 3-week-old striatal membranes, membranes from adult animals did not enhance the maturation of DA neurones, it can be suggested that the membrane DA factor disappears once DA innervation is complete. As with nerve growth factor, the synthesis of the DA factor by striatal cells could be reinitiated after partial or complete removal of DA afferent fibres in adult animals. Such a mechanism could explain the reinnervation of deafferented adult striatal neurones by mature or grafted embryonic DA cells¹¹⁻¹³.

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***In vivo* release of glutamate and aspartate following optic nerve stimulation**

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Glutamate (Glu) and aspartate (Asp) are considered to be major excitatory neurotransmitters in the central nervous system¹, but since the Jasper and Koyama report², the release of endogenous Glu or Asp on stimulation of an appropriate pathway *in vivo* has received little attention. They have been proposed as transmitters in the optic nerve of the pigeon (*Columba livia*)³⁻⁷. We now report that, using a push-pull cannula technique, the concentrations of Glu, Asp, glycine (Gly) and γ -aminobutyric acid (GABA) have been determined by mass fragmentography in perfusates from the optic tectum on electrical stimulation of the optic nerve or of the midbrain nucleus isthmi, pars parvocellularis (Ipc). Optic nerve stimulation markedly increased the content of Glu and Asp in the perfusate whereas stimulation of the nucleus Ipc was ineffective. The amount of GABA collected was increased only during Ipc stimulation. We conclude that Glu, Asp or related substances are involved in the transmission of the pigeon optic nerve terminals.

Glu and Asp have been proposed as transmitters in the pigeon optic nerve on the basis of the following evidence. Degeneration of the optic nerve results in a decreased content of Glu (50%) and Asp (40%) in the terminal layers of the optic nerve in the tectum⁶. The high-affinity uptake of Glu is reduced by 40–50% after optic nerve degeneration⁴. The degeneration of retino-tectal neurones has a protective effect on kainic acid histotoxicity in the optic tectum⁷. ³H-D-Asp, used as a possible false transmitter for Glu and Asp, is transported retrogradely and anterogradely in a subpopulation of optic nerve fibres⁵. Furthermore, after intraocular injection of ³H-D-Asp, the activity, which migrated anterogradely to the tectum, can be released by depolarization with 47 mM potassium in a Ca-dependent manner⁵.

The role of excitatory amino acids in optic nerve terminals of the pigeon was tested further by investigating the effect of optic nerve stimulation on the release of Glu, Asp, GABA and Gly (see Fig. 1). Figure 2 shows the fractional release of these amino acids from the optic tectum. Electrical stimulation of the optic nerve (Fig. 2a) induced a significant (two fractions before stimulation compared with the following two) increase of Glu ($P < 0.005$), Asp ($P < 0.02$) and Gly ($P < 0.001$) in the collected fractions, whereas GABA remained at < 0.5 pmol per fraction. In contrast, stimulation of the nucleus Ipc (Fig. 2b) did not significantly affect the amount of Glu and Asp in the fraction, but increased that of GABA and Gly ($P < 0.005$). The amino acid levels were also usually increased in the fraction immediately following the stimulation period.

These results suggest that Glu and Asp are released on stimulation of the optic nerve fibres and that they might be involved in at least part of their transmission. It cannot be excluded, however, that the release originates from elements which are postsynaptic to the optic nerve terminals and are indirectly activated by the stimulation. Nevertheless, the direct involvement of retino-tectal fibres is supported by the affinity of a population of these neurones for D-Asp (ref. 5). Alternatively,

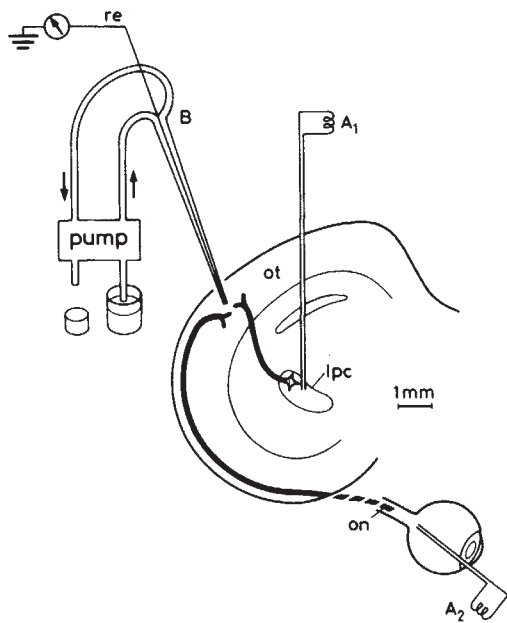


Fig. 1 Frontal section of the pigeon optic lobe showing the experimental set up. The experiments were performed on 10 adult pigeons anaesthetized with Equithesine⁹. Bipolar stimulating electrodes were implanted in the optic nerve (on) papilla (A₂) and the nucleus Ipc⁹ (A₁). Square wave pulses of 1–2 mA, 0.2 ms duration and at a frequency of 40 Hz were delivered for 5-min periods. The placement of the electrodes was controlled by recording evoked potentials (re) from the optic tectum (ot). The push-pull cannula (B) consisting of two parallel tubes (each 0.3 mm in diameter) was inserted in the superficial layers of the optic tectum. Twenty minutes after the onset of the perfusion ($12 \mu\text{l min}^{-1}$), 5-min fractions were collected. Three 5-min rest periods were intercalated between each stimulation period.

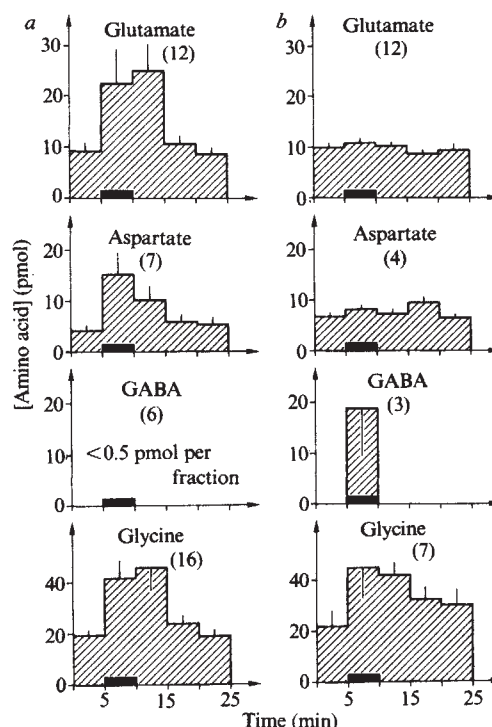


Fig. 2 *In vivo* release of amino acids in the optic tectum induced by electrical stimulation of two afferent neural pathways. Glu, Asp, GABA and Gly contents ($\pm$ s.e.m; number of determinations in parentheses) in perfusate fractions collected every 5 min through a push-pull cannula were determined by a mass fragmentographic method described by Wolfensberger and Amsler¹³. Stimulation (indicated by black bar) was applied to optic nerve (a) or nucleus Ipc (b).

the changes in amino acid levels might be related to a metabolic alteration induced by the stimulation. An argument against this is the fact that stimulation of the Ipc-tectal pathway, which has been shown to end predominantly in the same tectal layers as the optic nerve³, did not influence the release of Glu and Asp from the optic tectum. Finally, the evidence presented would also be consistent with the idea that Glu and/or Asp are metabolites of the released transmitter(s).

The release of Gly on optic nerve stimulation is difficult to interpret. It seems unlikely that it originates from retino-tectal fibres, as Henke *et al.*⁴ observed no decrease in the high-affinity uptake of Gly or in Gly levels after retinal ablation⁶. Gly could be released by the glycinergic Ipc-tectal pathway^{8,9} either presynaptically or by activation of the retino-tecto-Ipc-tectal circuit^{3,9}, although one wonders why GABA was not similarly affected. Intra-tectal glycinergic elements could also be indirectly activated by the optic nerve stimulation.

There is strong evidence that GABA is a transmitter in neuronal elements of the optic tectum^{10–12}. Following ³H-GABA injection into the tectum, a portion of the Ipc nucleus and of intrinsic tectal neurones were labelled; Gly and other amino acids did not label these neurones¹⁰. Taking this as evidence for GABAergic neurones, the GABA release observed in the present study may originate from some Ipc-tectal fibres and/or from intra-tectal neurones.

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Physiological evidence that the 2-deoxyglucose method reveals orientation columns in cat visual cortex

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A method for measuring local rates of glucose uptake in mammalian brain tissue^{1,2} has been used to study monkey cortex receiving visual stimulation through one eye³. The resulting pattern of glucose uptake resembled that of the ocular dominance columns, identified by several independent methods in the cortex^{4–6}. In addition, the pattern of glucose uptake produced by binocular viewing of stripes of a single orientation^{7–12} was consistent with the arrangement of orientation columns expected from the sequence of preferred orientations of single cells recorded in the visual cortex^{4,13–16}. It was therefore assumed that the columns of glucose uptake revealed in the studies of orientation selectivity correspond to columns of cortical cells selective for the orientation viewed. Recent findings about the monkey visual cortex led us to question this assumption.^{17–20} We have now investigated this question in the cat by making microelectrode recordings to determine preferred orientation of cortical units at known positions within the visual cortex and then stimulating the same animals with a full-field pattern of stripes to measure the rates of glucose uptake at the positions of each of the cortical units. As in previous studies, preferred orientation shifted gradually and progressively along electrode tracks parallel to the cortical surface^{4,13–16} and a pattern of densely labelled columns was observed in the 2-deoxyglucose autoradiographs^{7–12}. The centres of densely labelled cortical columns contained cells selective for the stimulated orientation. Preferred orientation shifted gradually away from the stimulated orientation at sites progressively more distant from the centres of the columns. Control experiments revealed no columns after stimulation with a pattern of changing orientation. Thus the columns revealed by the deoxyglucose method do correspond to the physiological orientation columns.

The experimental procedure consisted of two consecutive steps carried out on five normal adult cats. During the first step the preferred orientation of single cells in the medial bank of area 17 was determined by microelectrode recording; during the second step a striped pattern was presented to the animal after intravenous (i.v.) injection of deoxyglucose.

Initial surgery was carried out under barbiturate plus halothane anaesthesia. Venous and tracheal cannulae were inserted and a small skull opening made to expose the most posterior portion of one cortical hemisphere. On completion of surgery, the cats were paralysed (gallamine triethiodide, 10 mg per kg per h), and barbiturate anaesthesia was supplemented by artificial ventilation with 75% nitrous oxide–25% oxygen. End-tidal CO₂ was maintained at 4% and body temperature at 38 °C.

We then started a horizontal penetration into the medial bank of the lateral gyrus using a lacquered tungsten microelectrode²¹. The microelectrode shaft had been electropolished to <50 µm in diameter, allowing us to record from a long sequence of neurones in a single 10-mm penetration with minimal damage to the surrounding brain tissue.

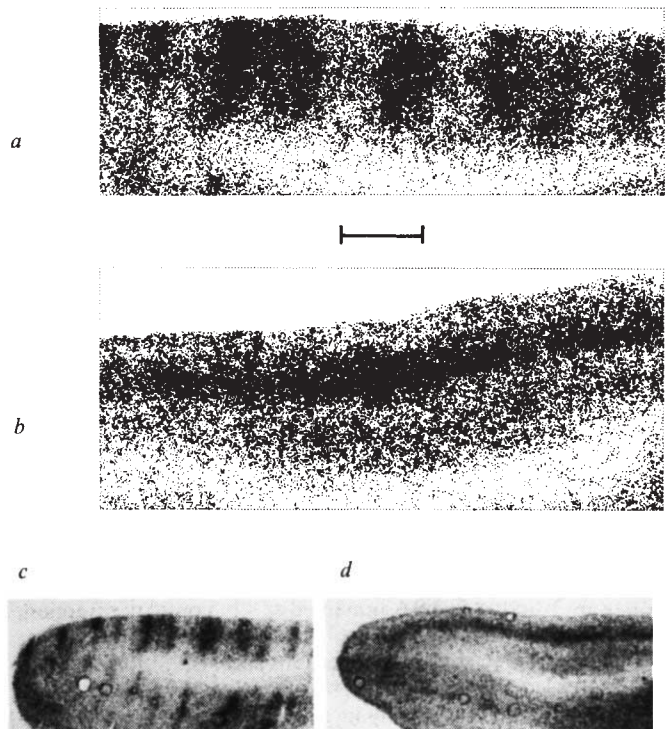


Fig. 1 Digitized radioactivity plots made from autoradiographs of parts of 20-µm horizontal sections of right visual cortex in two animals. *a*, Part of the medial bank of the lateral gyrus in area 17 of an animal that had viewed moving vertical stripes. Dark bands referred to as orientation columns run from white matter to cortical surface. *b*, Same area in a control animal that had viewed stripes of all orientations. No radial zones of increased radioactivity are visible. Up is medial, right posterior. *c*, Photograph of autoradiograph scanned in *a*. *d*, Photograph of autoradiograph scanned in *b*. Calibration bar is 1 mm for *a* and *b*, 3 mm for *c* and *d*.

The eyes were refracted and then aligned with a Risley prism by superimposing the receptive fields of a binocular unit. The areae centrales were plotted onto the tangent screen at 57 cm distance. Beginning 4–6 mm from the entry point, single cells from area 17 were recorded approximately every 100 µm. The preferred orientation of each cell was determined with a hand-held projector. An irregular sequence of electrolytic microlesions was made at ~1-mm intervals along the track by passing –4 µA for 4 s.

The penetration was terminated after studying about 40 single units. With the microelectrode kept in its final position, we injected an i.v. pulse of 100 µCi per kg ¹⁴C-2-deoxyglucose (Amersham, CFA.562) and exposed both eyes to a striped pattern moved perpendicular to the orientation of the stripes. This pattern consisted of white stripes 0.5–8.0° wide, separated by a similar spacing. It was swept in both directions at velocities increasing logarithmically from 0.3 to 20° s^{–1} during each 15-s period. We made sure that this pattern always covered at least the central 30° of the cat's visual field. In four of the animals, the pattern remained vertically oriented, while in one control animal the pattern was rotated between sweeps to each of 12 orientations, 15° apart.

After 45 min of exposure, the animals were given an overdose of barbiturate and were perfused with phosphate buffer followed by a buffered 4% solution of paraformaldehyde. The brain was then removed and blocks from cortical tissue cut and sunk slowly into Freon at –80 °C. The physiological experiment took 12–20 h; the whole procedure after killing the animal took 12–18 min.

Horizontal sections of the hemisphere containing the electrode track were cut at 20 µm in a cryostat, picked up on cover glasses, dried quickly on a hotplate at 70–80 °C and exposed along with a set of radioactive plastic standards (Amersham, 196363) to Kodak SB5 X-ray film for 10–15 days. The sections

were then stained with cresyl violet for reconstruction of the electrode track.

A flatbed autodensitometer²² measured optical density over areas of interest on autoradiographs containing the electrode tracks using a $32 \times 32 \mu\text{m}$ spot moved in $32\text{-}\mu\text{m}$ steps. Either 1,000 or 10,000 digitized optical density values per mm^2 were stored for each section together with the optical densities of the radioactive standards. This method allowed us to convert the optical density at any position on the section into a radioactivity level. Such radioactivity levels are linearly proportional to the rates of glucose uptake². Using a PDP11/23 computer we constructed a two-dimensional picture of the distribution of radioactivity which had produced each autoradiograph. Linear scans of such a picture made along the course (indicated by microlesions) of the electrode penetration quantitatively displayed the radioactivity as a function of electrode position.

Figure 1a shows a radioactivity scan of part of a horizontal section through area 17 from an animal that had viewed vertically oriented stripes. A pattern of dark and light bands extending mostly perpendicular to the brain surface is evident throughout all layers of grey matter. A similar scan taken from the brain of the control animal which had viewed stripes of all orientations is presented for comparison in Fig. 1b. No columnar variation in labelling density could be discerned, although differences in the labelling density between the laminae are evident. Autoradiographs from four additional control animals similarly disclosed no columnar variation in labelling density.

In most cases, the electrolytically marked locations in the brain were visible on the autoradiograph (as a reduction in the radioactivity over a distance of $50\text{--}150 \mu\text{m}$ along the track) as well as on the stained section. We were therefore able to locate recording sites by interpolation of the micrometre readings. Figure 2 shows examples of the finding that when a penetration crossed a labelled band, the preferred orientations of the cells recorded in this location were usually close to the vertical (the stimulated) orientation. On the other hand, lightly labelled portions of the electrode track usually matched recording sites of cells driven best by stimuli closer to the horizontal. A few cells (such as the second and third cells to the left of the third lesion) were exceptions to this general pattern. Some exceptions might be due to extracellular microelectrodes recording from units whose cell bodies are located at some distance from the electrode tip, perhaps in another column. The following analysis demonstrates that, despite these exceptions, the relationship between preferred orientation and labelling density at recording sites is highly significant.

A total of 10 labelled columns, either 2 or 3 in each of the four experimental animals, were both cut in cross-section (the cortical surface lay normal to the plane of section) and traversed by our electrode tracks. For example, three of these columns are to the left of the third lesion in Fig. 2b. To the right of this lesion, the cortex is cut obliquely, as shown in a Nissl-stained section (not illustrated) and by the failure of the labelled deoxyglucose bands to extend all the way from layer II to the white matter.

Within the parts of the electrode penetrations that crossed these 10 labelled bands, a total of 65 units were recorded. Figure 3 shows the relationship between the preferred orientation of these cortical units and the deoxyglucose labelling densities at their recording sites. For this analysis, preferred orientations (dashed line in Fig. 3a) and radioactivity levels (solid line in Fig. 3a) at recording sites were plotted in groups according to the distance of each recording site from the centre of the nearest labelled band. In effect, the analysis of Fig. 3a first superimposes the deoxyglucose columns at their centres to form an average column. Average preferred orientation and average radioactivity level are then plotted as a function of distance from the centre of this average column. The analysis confirms the results of earlier studies¹⁴⁻¹⁶—preferred stimulus orientation changes linearly with distance from the centre of the orientation column ($R = 0.86$, Fig. 3a). Figure 3a also shows the decrease in average radioactivity level with distance from the centre of the column. The data from Fig. 3a are replotted in Fig. 3b to show

that the radioactivity level is monotonically related to the preferred orientation ($R = 0.92$).

In the experiments using the 2-deoxyglucose method, labelled bands were seen in the visual cortex of cats that viewed vertical stripes. When animals viewed stripes of all orientations, no such bands were observed. Microelectrode recording showed that the labelled bands corresponded to vertical orientation columns.

These findings support the central assumption made in previous studies of orientation selectivity in cat visual cortex using the 2-deoxyglucose method⁷⁻¹². They suggest that the deoxyglucose method should be useful for further studies of columnar systems in cat cortex. The present findings do not show whether orientation columns in the cat's visual cortex are discrete entities; the results are consistent with such a hypothesis but are also consistent with the notion that preferred stimulus orientation varies continuously across the cortical surface.

In the monkey's visual cortex, labelled columns have been observed¹⁹ in conditions of visual stimulation similar to those which produced no labelled columns in our control cats. It has not yet been reported whether the labelled columns seen in the

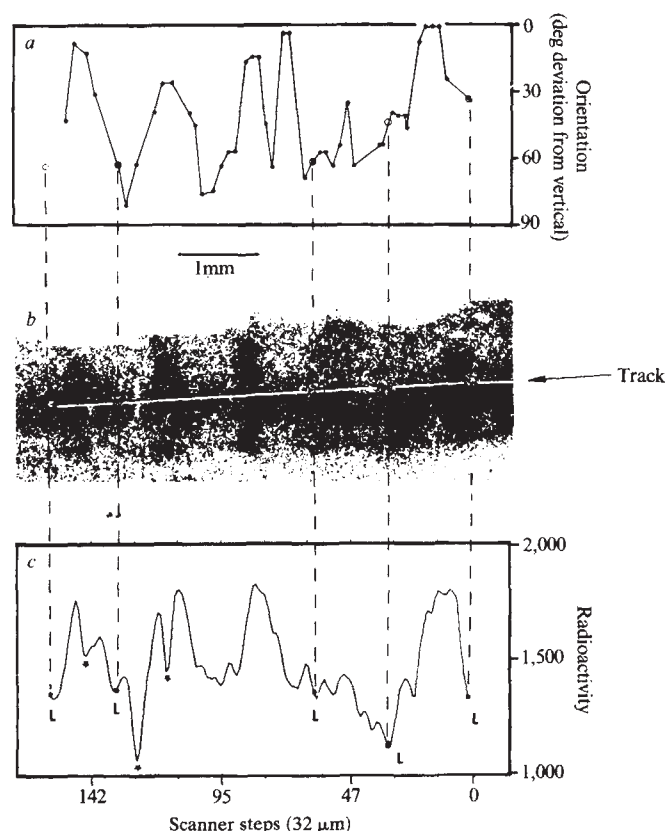


Fig. 2 Preferred orientation of single cells (a) and radioactivity (proportional to glucose uptake) (c) plotted as a function of distance (abscissa) along a microelectrode track (b) into area 17 of the visual cortex. Track starts at the right-hand side (arrow in b) and runs along the white line. a, Position of each dot gives the deviation of a single cell's preferred orientation from vertical, the orientation of the striped pattern used in the deoxyglucose experiment. Thus vertically oriented cells are represented by dots at the peaks of the graph, horizontally oriented cells at the troughs of the graph, and the two diagonals in between. b, Display of radioactivity made from autoradiographs of two adjacent $20 \mu\text{m}$ horizontal sections from area 17 of a cat's visual cortex. In the autoradiographs of these sections, the whole course of the penetration could be traced by means of microlesions seen at the interruptions of the white line. c, Radioactivity (in arbitrary units) along the electrode penetration. 'L' marks troughs corresponding to light areas in the autoradiograph caused by microlesions. '*' Marks troughs caused by fractures in the tissue. The vertical dashed lines connect positions of microlesions throughout the figure (open circles in a, interruptions of the white line in b, 'L' in c). It is apparent that peaks in the graph a (vertically oriented cells) match areas of high radioactivity levels (b, c) and vice versa.

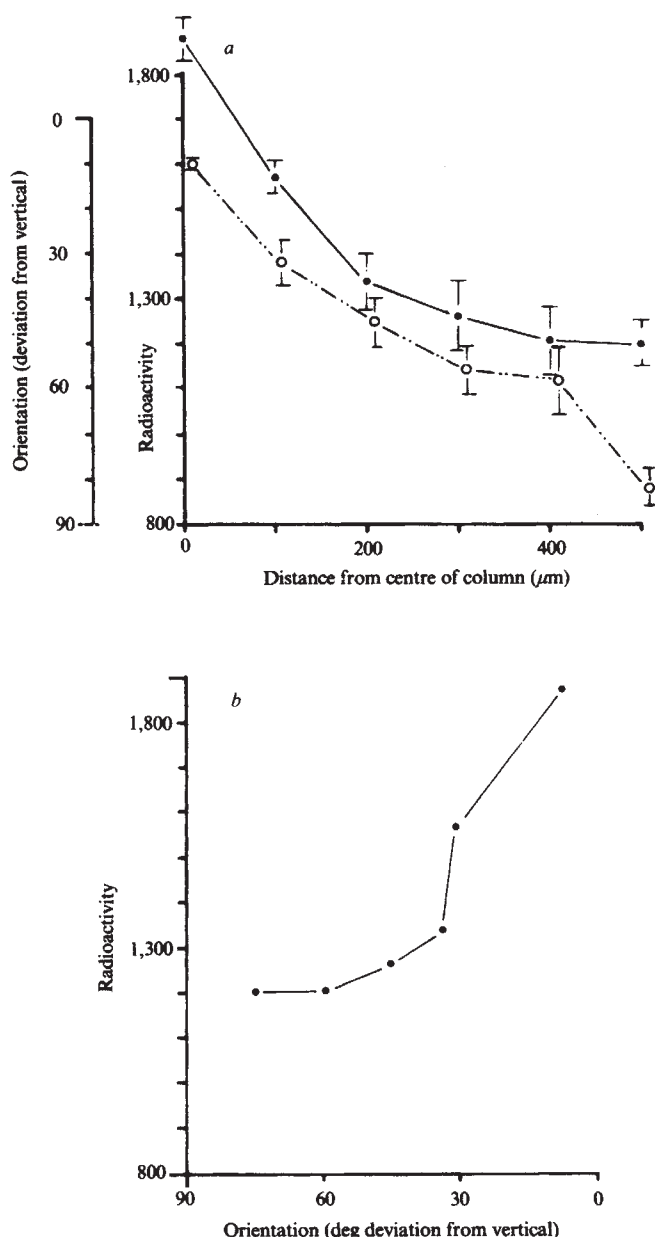


Fig. 3 *a*, Deviations of cells' preferred orientations from vertical $\pm$ s.e.m. (left ordinate, dashed line, ○) and radioactivity $\pm$ s.e.m. (right ordinate, solid line, ●) as functions of distance (abscissa) from the centres of 10 superimposed dark bands (orientation columns) in four experimental animals. *b*, Data from *a* plotted to show relationship between preferred orientation of recorded units and radioactivity (proportional to glucose uptake rate) at recording sites.

unstimulated monkey have the same range of densities as those seen in monkeys stimulated with patterns of single orientation. With this question unresolved, it would not be safe to extend the results of the present study to the monkey.

It will be of interest to determine to what extent the relationship between orientation specificity and the cortical metabolic pattern seen in the present study is based on a more general relationship: is neuronal firing the primary determinant of metabolic activity in the cortex? Experiments in progress, quantitatively comparing the neuronal discharge frequency elicited by visual stimuli with glucose uptake produced by these same stimuli,²³ will answer this question.

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Immunocytochemical localization of enkephalin and substance P in retina and eyestalk neurones of lobster

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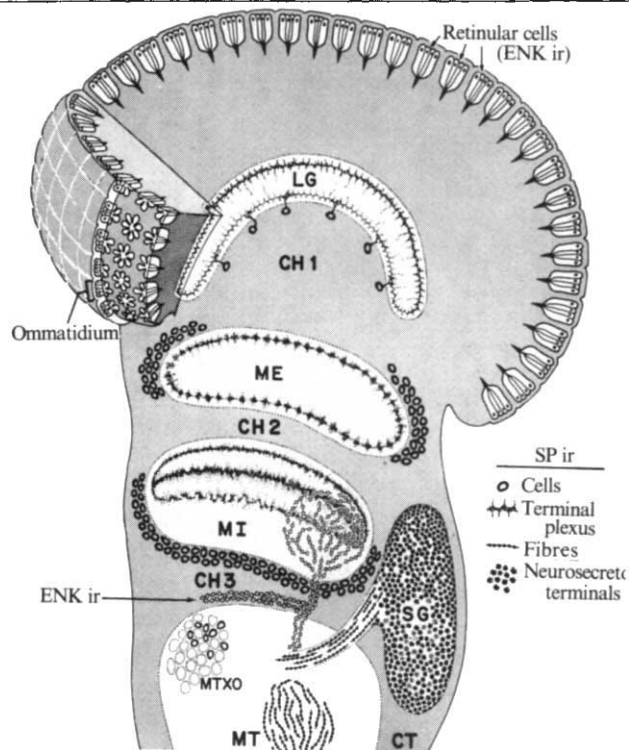
Enkephalin and substance P are two peptides found in vertebrate nervous systems that have been intensively studied with regard to cellular distribution and receptor mechanisms^{1–3}. Invertebrate nervous systems, which are extremely useful in elucidating general principles of neural functions^{4–6}, can also be used as models for the analysis of peptidergic mechanisms^{7,8}. The accessibility, regularity, small number and relatively large size of the neural components of one such system, the crustacean compound eye, have facilitated the accumulation of detailed information about their anatomy^{9–11} and physiology^{12–14}, and provided insight into basic principles of intercellular communication^{12–14}. We report here that immunoreactivity of enkephalin and substance P can be localized to specific neural elements in the visual system of the spiny lobster, *Panulirus interruptus*. Our observations suggest that this highly organized, simple *in vivo* system may be a convenient model to evaluate the cellular mechanisms of these peptides. Although several neuropeptides have been localized in vertebrate retinal interneurons¹⁵, our results are the first to detect a known peptide in primary photoreceptors.

Lobster eyestalks were fixed in paraformaldehyde at 4 °C, and stored at 4 °C for 1–3 days until sectioned. Frozen sections (20 μm) were collected on gelatin-coated slides and incubated at 4 °C for 15–18 h with either anti-rabbit Leu-enkephalin antiserum, anti-rabbit β-endorphin antiserum, or monoclonal anti-rat substance P antibody. After rinsing, primary antibodies were localized by indirect fluorescence (see Fig. 1 legend for details).

Specificity of immunoreactivity was evaluated by incubating tissue sections with anti-substance P antiserum absorbed with synthetic substance P or anti-enkephalin antiserum absorbed with synthetic Leu-enkephalin (see Fig. 1 legend for details). Further sections were incubated with phosphate-buffered saline (PBS) or non-immune serum instead of primary antibody. Given the limitations of the immunocytochemical approach, however, the immunoreactive staining observed may also be due to

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Fig. 1 Distribution of enkephalin (ENK) and substance P (SP)-like immunoreactivity (ir) in the lobster eyestalk. The optic components include the retina, which contains the photoreceptor units, the ommatidia. Each ommatidium contains seven primary photoreceptors, the reticular cells¹¹. The retina extends from the distal end of the reticular cells to the distal border of the lamina ganglionaris. Information from the retina is relayed to the four optic neuropiles: the lamina ganglionaris (LG), the medulla externa (ME), the medulla interna (MI) and the medulla terminalis (MT)^{9,11}. These structures are connected to each other by fibres forming the first (CH1), the second (CH2) and the third (CH3) chiasm. The eyestalk also contains the MTXO-sinus gland (SG) neurosecretory complex. Only enkephalin- and substance P-immunoreactive cells and fibres are shown. Enkephalin immunoreactivity (ENK ir) is present in each reticular cell. Fibres (○—○) in CH3 and MI also contain enkephalin immunoreactivity (thin arrow). Substance P immunoreactivity (SP ir) is present in all other cells, fibres and terminal plexuses shown. In the lamina, medulla externa and medulla interna, substance P-like immunoreactivity was confined exclusively to neurones whose fibres extend horizontally. These neurones correspond to the neurosecretory cells of the lamina¹⁶, Hanström's cell type 11 in the medulla externa¹⁰ and Hanström's cell type 15 in the medulla interna¹⁰. Substance P immunoreactivity is also present within a subpopulation of cells, fibres and neurosecretory terminals within the MTXO-sinus gland complex (see text for details). CT, connective tissue. Eyestalks were quickly dissected under normal room illumination and fixed in 4% paraformaldehyde–0.5% PBS, pH 7.4, at 4°C. After 10–18 h, the tissue was transferred to 30% sucrose–PBS at 4°C until sectioning in a Harris cryostat in transverse or longitudinal planes. Anti-Leu-enkephalin antiserum A-206 (donated by R. J. Miller) and anti- β -endorphin antiserum (Bendo-2, donated by R. Benoit and R. Guillemin) were both diluted 1:1,000, with 1 mg ml⁻¹ bovine serum albumin (BSA). Anti-SP antibody MS-035 (Sera Lab) was diluted 1:100. All antibodies were diluted in 0.3 M PBS–0.3% Triton X-100. Anti- β -endorphin and anti-Leu-enkephalin reactivity were localized using goat anti-rabbit IgG–fluorescein isothiocyanate (FITC) (Antibodies, Inc., California) and anti-substance P was localized with rabbit anti-rat IgG–FITC (Miles), both diluted 1:25 in 0.3 M PBS–0.3% Triton X-100. After rinses in PBS, slides were air-dried and a coverslip added in paraffin oil. The sections were examined using a Zeiss Universal fluorescence microscope with a Xenon epi-illumination light source (XBO-150) and equipped with a Zeiss filter pack (48–77–09–9903). The sections were photographed with a Nikon camera on Kodak Ektachrome (Tungsten 160 ASA) or Tri-X (400 ASA) pan film. To test for specificity of the immunoreactive staining, anti-substance P antibody (1:100) and anti-enkephalin antibody (1:1,000) were absorbed with synthetic substance P (Sigma) or synthetic Leu-enkephalin (Sigma), respectively, at concentrations of 0.1, 1.0, 10 and 100 μ M at 4°C overnight. Absorbed antiserum, antiserum which had been diluted and stored overnight at 4°C, PBS, or non-immune serum, were used instead of freshly diluted primary antibody on control sections. All staining was abolished by absorption of anti-enkephalin with enkephalin at all concentrations used. Substance P immunostaining was totally abolished at 10 and 100 μ M and partially blocked at 0.1 and 1 μ M.



peptides that are structurally related to the immunogenic peptides (for example, substance P: eleudoisin or physalaemin).

We examined the neural structures of the eyestalk, including the retina, the four optic neuropiles (ganglia) and the distal portions of the optic nerve. The distribution of substance P- and enkephalin-immunoreactive cell bodies and fibres within the eyestalk is summarized in Fig. 1.

Prominent enkephalin-like immunoreactivity was observed in all the primary photoreceptors, the reticular cells. Sectioning of the retina in different planes revealed that the immunoreactivity was present in all seven reticular cells (Fig. 2a), throughout each perikaryon (Fig. 2b), except for the region of the nucleus which was unstained (top inset, Fig. 2b). All enkephalin immunoreactivity was abolished by absorbing the antibody with 0.1 μ M synthetic Leu-enkephalin (bottom inset, Fig. 2b).

Enkephalin-like immunoreactivity was also observed in fibres which pass in a centrifugal direction through the medulla terminalis and terminate in a plexus of fine varicose fibres in the medulla interna (see Fig. 1). As all fibres from reticular cells are believed to terminate in the lamina ganglionaris¹⁶, the enkephalin-immunoreactive fibres terminating in the medulla interna must represent a second type of peptide-containing cell (J.R.M. and J.F.M., in preparation).

Substance P-like immunoreactivity was observed in each of the four optic neuropiles. Fibres were consistently observed in two well defined layers of the lamina ganglionaris; some of the fibres could be traced to substance P-like immunoreactive cell bodies located in the proximal fringes of the lamina (Fig. 1). Hamori and Horridge¹⁶ described presumptive neurosecretory cells with a similar location, which gave rise to horizontal fibres virtually identical to these substance P-immunoreactive fibres in the lamina ganglionaris.

We also found substance P-like immunoreactive fibres arranged in a discrete manner in two identified synaptic layers of the medulla externa (Fig. 2c) and in three such layers of the medulla interna. Of the several identified cell types associated with these two optic neuropiles^{9,10}, only one neuronal type in each medulla contained substance P-like immunoreactivity (Fig. 1).

In the medulla terminalis, we observed two additional types of substance P-immunoreactive fibre: a diffuse plexus in the central portion of the neuropile, reflecting the less stratified organization of the medulla terminalis, and a second group of substance P-like immunoreactive fibres passing through the distal part of the medulla terminalis and ending as large spherical terminals in the sinus gland (Fig. 2d). A small subpopulation of cells in the medulla terminalis X organ (MTXO) contained substance P-like immunoreactivity (Fig. 1). The MTXO-sinus gland neurosecretory complex is believed to be an evolutionary analogue of the hypothalamic-hypophyseal system^{17,18}. Finally, substance P-like immunoreactive fibres were observed to pass through the optic nerve.

No β -endorphin immunoreactivity was detected in the eyestalk. The localization of somatostatin immunoreactivity in cells and fibres of the lobster eyestalk will be described elsewhere (J.R.M. and J.F.M., in preparation). The distribution of enkephalin- and substance P-like immunoreactivity is now being studied in the crayfish (*Procambarus clarkii*), a closely related crustacean species, and an extremely similar pattern has been observed. However, in the crayfish, enkephalin-like immunoreactivity is clearly observed in fibres that pass between the primary photoreceptors and the lamina ganglionaris and in terminals throughout the lamina. In addition, substance P-like immunoreactive fibres have been observed to cross the first optic chiasm.

The detection of enkephalin-like immunoreactivity in the reticular cells suggests a possible neurotransmitter function for enkephalin or a closely related peptide in the primary photoreceptors of the lobster eye. Exogenous enkephalin and opiates have been found to interact with substance P-containing primary sensory afferents in the substantia gelatinosa of the mammalian spinal cord^{19–21}. A similar, though by no means identical, relationship is suggested by our demonstration that enkephalin and substance P immunoreactivity is present in synaptically related cells, the reticular and 'neurosecretory' cells, of the lobster visual system. Electron microscopic studies have shown that, although most reticular cells terminate on the ganglion cells of the lamina, many form axo-axonic synapses on

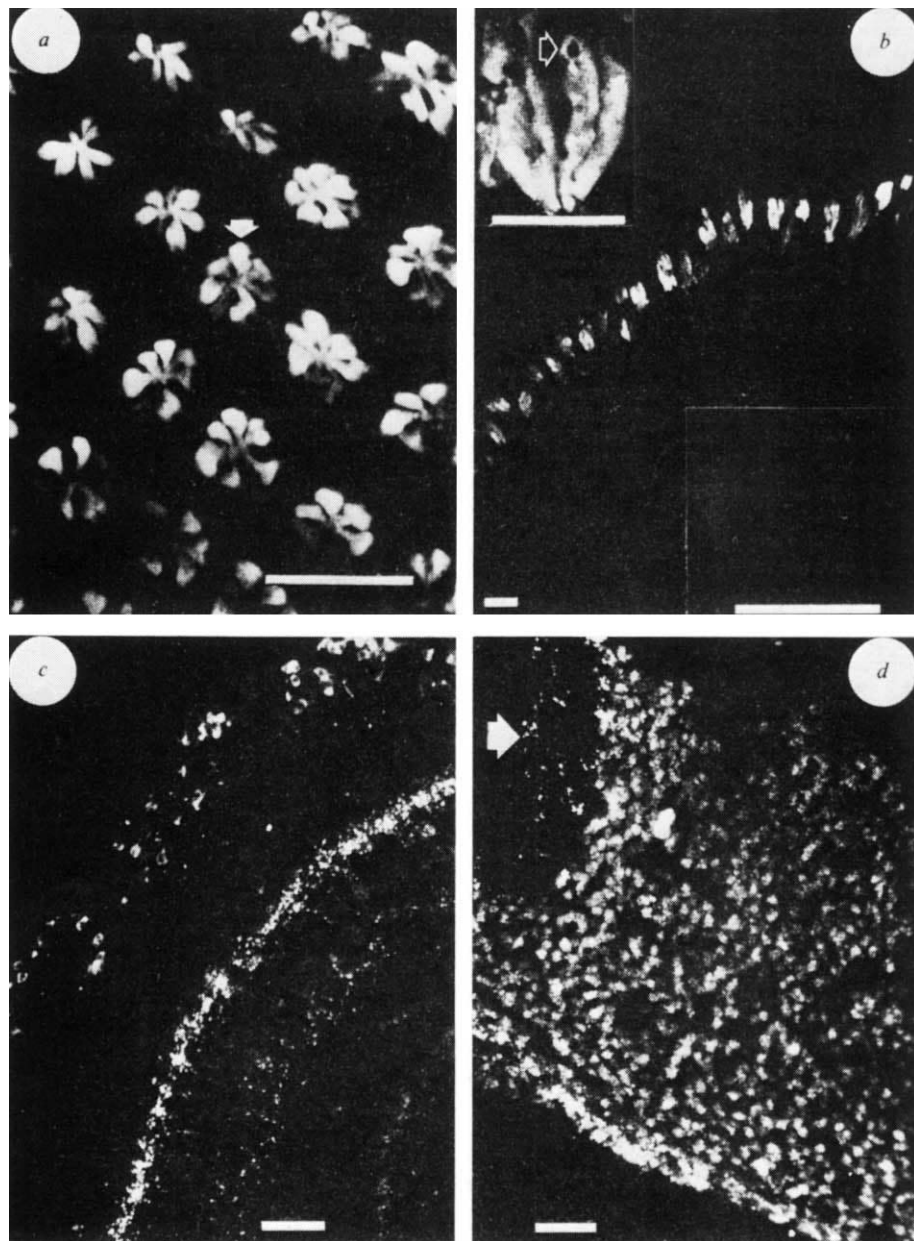


Fig. 2 Enkephalin- and substance P-like immunofluorescence in the lobster eyestalk. Calibration bar, 50 μ m. *a*, Transverse section of retina showing that each reticular cell (arrow) in each ommatidium contains enkephalin-like immunoreactivity. *b*, Longitudinal section of retina showing enkephalin immunoreactivity throughout the length of perikarya of reticular cells. Top inset: higher magnification of reticular cells (the arrow indicates an unstained nucleus). Bottom inset: longitudinal section of retina showing unstained photoreceptors incubated with anti-enkephalin antiserum absorbed with 0.1 μ M enkephalin. *c*, Substance P-like immunoreactivity in a longitudinal section of the medulla externa. Immunoreactive cells correspond to a subset of Hanström's cell type 11 (ref. 10). Note the laminar pattern of substance P-immunoreactive terminals. *d*, Immunoreactive fibres (arrowhead, top left) and neurosecretory terminals in the sinus gland, which is a neurohaemal organ consisting of neurosecretory terminals with cell bodies located in the MTXO²⁶. The immunoreactive material in the sinus gland seemed to be contained within large spherical granules resembling those of the neurosecretory terminals described elsewhere²⁷.

the 'neurosecretory' cell fibres. In turn, the 'neurosecretory' cells themselves provide input to the ganglion cells²². Thus, the lamina ganglionaris of the lobster may prove to be a useful system for studying possible naturally occurring sequential interactions between enkephalin and substance P.

Finally, our results provide additional support for the presence in invertebrates of immunoreactivity to two neuroactive peptides first detected in and isolated from mammals.

Recently, enkephalin-like immunoreactivity has been localized in specific neurones of the leech²³ and earthworm²⁴, and has been detected by radioimmunoassay in the locust²⁵. Our findings may therefore expand the opportunities for studying the mechanisms of action of mammalian neuropeptides by exploiting less complex invertebrate nervous systems.

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Conserved amino acid sequence of a neuropeptide, the head activator, from coelenterates to humans

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We have recently isolated and sequenced a neuropeptide from the freshwater coelenterate hydra (*Hydra attenuata*), which was named head activator because it controls head-specific growth and differentiation processes¹⁻⁴. The head activator is an undecapeptide of molecular weight 1,142, with the sequence pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe. We have also previously purified a peptide from mammalian brain and intestine which has similar biological and chemical properties to those of the head activator from hydra^{5,6}. Here, we present evidence that a peptide of identical amino acid sequence to the head activator can be isolated from human hypothalamus, bovine hypothalamus and rat intestine. This is the first example of a neuropeptide conserved structurally intact from a most primitive nervous system to the advanced nervous system represented in humans.

Head activator, as assayed by its biological effect on hydra, was found in brain and intestine of cow, pig, rat and human^{5,6}. The hypothalamus of an adult rat contained 10⁵ hydra equivalents, the intestine 10 times more. Cow and pig contained comparable amounts per animal. The best source was human, where 10⁸ hydra equivalents were found in the hypothalamus of an adult male⁶.

We first extracted the head-activator-like peptide from the intestines of 3,000 1-2-month old rats; the peptide was purified in three batches of 1,000 animals each. The rats were killed in a CO₂ atmosphere, and the intestines from the pylorus to the last thickening of the large intestine were dissected and submerged immediately in 20 l of cold methanol containing the protease inhibitor phenylmethylsulphonyl fluoride (0.05%). The purification was similar to that described for the head activator from hydra¹. After homogenization and centrifugation the methanol supernatant was condensed and extracted with petroleum ether and chloroform to remove lipids. The aqueous phase was subjected to ion-exchange chromatography on Sephadex DEAE-A-25 (batchwise) and to molecular sieving using Sephadex G-10 and Biogel P-2 alternately, followed by reverse-phase chromatography with the octylsilyl silica gel LiChrosorb RP-8 with various organic solvents as eluents in low and high pressure conditions. The location of the desired substance was determined by assaying the biological activity as stimulation of bud growth in hydra³, and in later stages of purification, by first searching for the typical amino acid pattern of the head activator (after hydrolysis and separation of the dansylated amino acids by TLC⁷) before assaying the biological activity.

The head activator from bovine hypothalamus was obtained from a side fraction of a neurotensin isolation by R. Caraway (then at S. Leeman's laboratory), in which bovine hypothalamus were chromatographed on a 20-l Sephadex G-25 column⁸. A fraction eluting later than neurotensin and substance P (15.5-18 l) contained head-activator activity. After lyophilization this fraction was extracted with methanol and treated as above.

Head activator was also isolated from the hypothalamus of two men aged 52 and 65 yr. The tissues were excised 20 h post mortem and stored in cold methanol before homogenization. After extraction with petroleum ether and chloroform the aqueous phase was chromatographed on a 250-ml Sephadex G-10 column (5.5 × 13 cm) using distilled water as eluent. The fraction from 500 to 1,000 ml was concentrated and desalted on a LiChrosorb RP-8 column under low pressure¹.

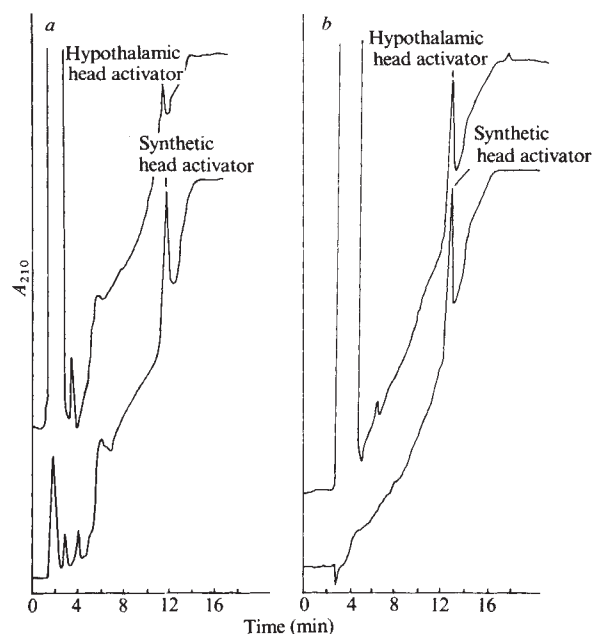


Fig. 1 Reverse-phase HPLC of the head activator from human hypothalamus (upper tracing) on a LiChrosorb RP-8 column, particle size 7 μ m, column size 250 × 4 mm. The lower tracing shows the absorbance of the synthetic head activator (500 pmol were injected). Elution was carried out with a gradient in: a, 40-60% methanol in 5 mM ammonium bicarbonate; b, 20-40% acetonitrile in 0.1% trifluoroacetic acid, for 10 min at a flow rate of 1 ml min⁻¹.

For the final purification of all three peptides, we used reverse-phase HPLC so as to obtain a homogeneous material and to compare the elution times of the peptides from the different origins. The extracts were first separated isocratically with 50% methanol in 5 mM ammonium bicarbonate, pH 7.8, and/or with 30% acetonitrile 0.1% trifluoroacetic acid. As final steps the peptides were subjected to gradient elutions using methanol or acetonitrile in the mobile phase. As shown in Fig. 1 for the peptide from human hypothalamus, the retention times in both systems are identical to those of synthetic⁹ or native¹ head activator from hydra. The retention times of the peptides from rat intestine and from bovine hypothalamus were also identical. Yields of pure peptide were 20 nmol from 3,000 rat intestines, 2 nmol from human hypothalamus and 10 nmol from the bovine hypothalamic fraction.

The peptide was then hydrolysed in 6 M HCl for 24 h and amino acid identification carried out after dansylation by two-dimensional TLC⁷. For a quantitative amino acid analysis we separated the dansylated hydrolysate on reverse-phase HPLC using both optical density and fluorescence for detection. The amino acid composition of the peptides of all three origins was identical with that of the head activator from hydra: Gly (2), Glu (1), Ile (1), Leu (1), Lys (1), Phe (1), Pro (2), Ser (1), Val (1).

As expected from the NH₂-terminal sequence of the head activator from hydra, no end group was found for the biologically active peptides. However, during the last purification steps we involuntarily produced a peptide with identical amino acid composition but different retention times on HPLC, which had no biological activity and glutamic acid as end group. The opening of the pyroglutamyl ring was probably due to the mild hydrolysis conditions of the HPLC buffers used. This peptide (Glu-peptide) could be converted into the active peptide by treatment with anhydrous trifluoroacetic acid, which results in a cyclization of the amino-terminal glutamic acid to pyroglutamic acid. The same peptide has been found during the isolation of the head activator from hydra¹.

Partial sequencing of the Glu-peptide from rat intestine by the consecutive dansyl Edman procedure¹⁰ showed the NH₂-terminal sequence to be Glu-Pro-Pro-Gly-Gly-, which is identical with that of the peptide from hydra. A further chemical

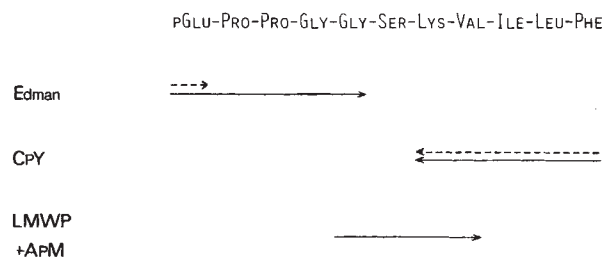


Fig. 2 Procedure for the sequence analysis of the head activator from rat intestine (—) and from human and bovine hypothalamus (---). Incubations with carboxypeptidase Y (CpY) were performed at an enzyme concentration of $100 \mu\text{g ml}^{-1}$ in 0.1 M ammonium acetate, pH 6.0. The incubation with low molecular weight protease (LMWP) was carried out at a concentration of $1 \mu\text{g ml}^{-1}$ in 0.05 M ammonium acetate, pH 7.6. For the subsequent digest with aminopeptidase M (APM) we used an enzyme dilution of $10 \mu\text{g ml}^{-1}$ in the same buffer. All enzymatic incubations were performed in $2 \mu\text{l}$ at 37°C . For end-group analysis and enzyme digests, 20–100 pmol of the peptide were used. For a consecutive Edman degradation 20–50 pmol per vial were sufficient.

degradation was impossible due to the strongly hydrophobic nature of the residual peptide¹. The COOH-terminal end was sequenced by kinetic studies with carboxypeptidase Y (CpY) (see Fig. 2). Using incubations of 2, 5 and 15 min, the COOH-terminal sequence of the Glu-peptide was shown to be -Lys-Val-Ile-Leu-Phe, identical with that of the head activator from hydra. Similarly, the biologically active pGlu-peptide was only degraded up to Ile which suggests that the Glu-peptide and the pGlu-peptide have very different conformations. The biologically active peptide was not affected by chymotrypsin or pyroglutamate aminopeptidase, confirming Phe as the carboxy-terminal amino acid and pGlu-Pro- as the amino-terminal sequence. Incubation with a low molecular weight protease isolated from *Astacus fluviatilis*¹¹ yielded two fragments and Gly as free amino acid. A subsequent kinetic digestion with aminopeptidase M revealed Ser-Lys-Val- as neighbouring sequences to Gly, followed simultaneously by Ile, Leu, Phe. From the carboxy- and amino-terminal sequences and from the overlap of a fragment after low molecular weight protease digestion, we deduced the following sequence for the biologically active peptide from rat intestine (Fig. 2): pGlu-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe. This sequence is identical with that determined for the head activator from hydra. Because of the limited amount of pure peptide available, only a part of the amino acid sequence of the hypothalamic peptides was determined. However, we found that the hypothalamic peptides and hydra head activator were identical in: (1) amino acid composition, (2) COOH-terminal sequence over five amino acids, (3) amino-terminal amino acid, (4) retention times on HPLC and (5) biological effects on hydra. These findings are taken as evidence that the peptides from human and bovine hypothalamus are also identical to the head activator from hydra.

We have synthesized the head activator and have shown that the synthetic and the native peptide are chemically and biologically identical⁹. We also found that deletion or addition of one or more amino acids, conversion of pyroglutamic acid to glutamic acid and derivatization of the ϵ -amino function of lysine, resulted in destruction of the biological activity on hydra⁹. We deduce from this that the structure of the head activator is very rigid, allowing little or no conformational change. In hydra, the first organism in the animal kingdom to possess a nervous system, the head activator is produced by nerve cells and is stored there in neurosecretory granules¹². In mammals the head activator could only be extracted from brain and intestine. The fact that other mammalian neuropeptides occur in brain and intestine has been interpreted to mean that they may have a common embryological origin¹³. Our findings with the head-activator peptide suggest that some of these do have phylogenetically a common neuronal ancestor.

The sequence of the head activator differs from those of all other biologically active peptides described so far. The only structural homology we have observed is with bradykinins, in the sequence -Pro-Pro-Gly-X-Ser- (ref. 14). The amino-terminal sequence of bradykinin is Arg-Pro-Pro-Gly-Phe-Ser-Pro-, that of the head activator pGlu-Pro-Gly-Gly-Ser-Lys-. As the function of the 'head activator' in mammals is unknown, it is not clear whether this structural homology implies a functional homology as well. From its early appearance in the developing mammalian embryo⁶ and in analogy to hydra⁴ one could postulate that the peptide has growth regulatory functions. Whether it has additional functions in the adult as transmitter or modulator remains to be elucidated. The synthesis of the peptide now provides a means of studying its function in the mammalian system.

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C2 synthesis by human monocytes is modulated by a nicotinic cholinergic receptor

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Receptors for the neurotransmitter acetylcholine have been found on several types^{1–9} of cell outside the neuromuscular system and exocrine secretory tissue. Thus cholinergic ligands have been shown to increase phytohaemagglutinin-induced transformation, RNA and protein synthesis and antibody-independent cytotoxicity in lymphocytes, and the IgE-mediated release of histamine, slow reacting substance of anaphylaxis (SRS-A) and eosinophil chemotactic factor of anaphylaxis (ECF-A) in mast cells. These effects were shown to be mediated by muscarinic receptors. However, recent evidence has suggested that nicotinic receptors are present on lymphoid cells^{8,10,11}. We report here that synthesis of C2, the second component of complement, by human monocytes is enhanced by the cholinergic ligands acetylcholine and carbamylcholine, and that this effect is mediated exclusively by a nicotinic receptor which is similar in pharmacological specificity, and antigenically related, to the nicotinic receptors of human skeletal muscle and the electroplaques of the electric ray, *Torpedo marmorata*.

The control of C2 synthesis by mononuclear phagocytes has been poorly defined to date¹². To investigate this issue,

monolayers of peripheral blood monocytes were prepared and cultured *in vitro* by a previously published technique¹³. Monolayers consisted of >95% monocytes as judged by nonspecific esterase staining, phagocytic activity and possession of Fc receptors¹⁴. In the culture conditions, monocytes did not synthesize C2 for 2 days but by the third day C2 was detectable in the culture supernatants. The concentration of C2 then rapidly increased until the sixth or seventh day, when the rate of increase slowed down and plateaued¹⁴. When acetylcholine or carbamylcholine was added to the cultures, the C2 concentration in the supernatants increased in a dose-dependent fashion (Fig. 1). Carbamylcholine was significantly more potent than acetylcholine ($P < 0.05$) at concentrations of 10^{-4} – 10^{-6} mol l⁻¹. Unlike these cholinergic ligands, pilocarpine had no effect on C2 production by monocyte cultures (Fig. 1).

The increased concentrations of C2 in the supernatants of acetylcholine- or carbamylcholine-treated monocyte cultures was due to enhanced synthesis of this protein as the addition of the protein-synthesis inhibitors, cycloheximide, puromycin or mitomycin C (all at 2.5 µg ml⁻¹) inhibited the effect of both ligands (Fig. 2).

The addition of the muscarinic antagonist atropine (10^{-4} mol l⁻¹) to the culture medium did not alter the acetylcholine- or carbamylcholine-augmented synthesis of C2,

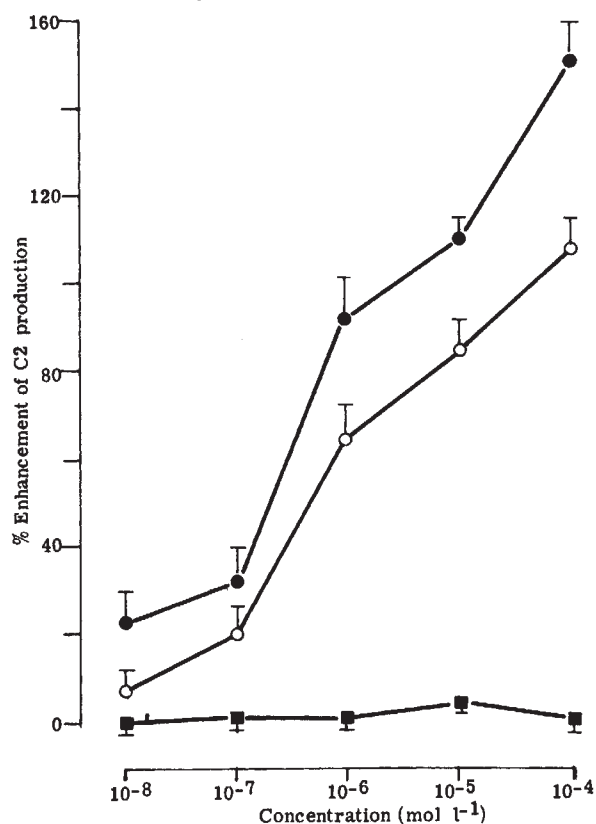


Fig. 1 Enhancement of monocyte C2 synthesis by the cholinergic ligands acetylcholine and carbamylcholine. Monocyte monolayers were prepared in Linbro multiwell tissue culture plates and cultured in the RPMI 1640 medium containing 20% heat-inactivated fetal calf serum in an atmosphere of 5% CO₂ in air¹³. On day 14 of culture, the supernatants were removed, the monolayers washed in warm medium and lysed in 2% SDS solution. The DNA content of the lysates was determined spectrofluorimetrically²⁰, and the C2 contents of the supernatants measured by stoichiometric haemolytic assay²¹ and expressed as effective molecules per µg DNA. The addition of acetylcholine (○) or carbamylcholine (●) to monocyte cultures produced a dose-dependent enhancement of C2 synthesis, whereas pilocarpine (■) was ineffective. % Enhancement of C2 synthesis = [(C2 content of treated culture - C2 content of control culture)/C2 content of control culture] × 100. Each point represents the mean of three experiments performed in duplicate and the horizontal bars represent the s.e.m.

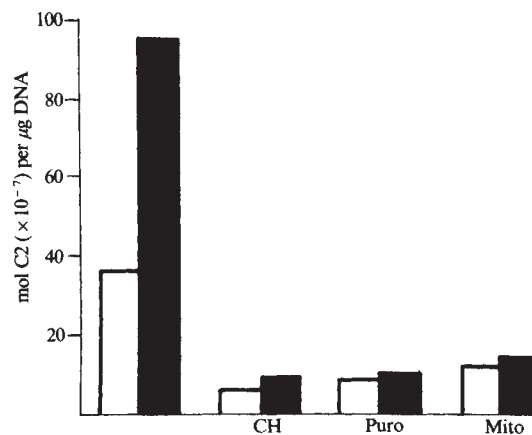


Fig. 2 Effect of protein synthesis inhibitors on C2 production. Monocytes were cultured in the absence (□) or presence (■) of acetylcholine. Culture conditions are as for Fig. 1. The addition of cycloheximide (CH), puromycin (Puro) or mitomycin C (Mito), all at 2.5 µg ml⁻¹, to the cultures inhibited C2 synthesis in both control and acetylcholine-treated cultures. C2 levels were measured on day 14 of culture. The results represent the mean of two experiments performed in duplicate. The protein-synthesis inhibitors were equally effective when added to carbamylcholine-treated cultures (data not shown).

whereas the nicotinic antagonists α -bungarotoxin (10^{-8} mol l⁻¹) or (+)tubocurarine (10^{-7} mol l⁻¹) abrogated the effect (Fig. 3). The concentration of nicotinic agonists (acetylcholine and carbamylcholine) used here is similar to those known to produce physiological responses in cultured muscle cells¹⁵, but identical doses of the muscarinic agonist had no effect. Similarly, low concentrations of the high-affinity nicotinic antagonists ((+)tubocurarine or α -bungarotoxin) produced potent inhibition whereas the muscarinic antagonist, atropine, had no effect even at a 1,000-fold higher concentration. These findings, together with the observation that pilocarpine, a ligand acting selectively at muscarinic receptors, has no effect on C2 synthesis (Fig. 1), demonstrated that the enhancement of C2 synthesis was mediated exclusively by nicotinic receptors.

Further studies revealed that the nicotinic receptors of human monocytes and skeletal muscle were antigenically similar. IgG was purified from the serum of a myasthenia gravis patient with a high titre of circulating antibodies to acetylcholine receptors (120×10^{-9} mol l⁻¹). When this IgG or its F(ab')₂ fragment was added to monocyte cultures, the effects of acetylcholine were abrogated, a result not observed when normal IgG or its F(ab')₂ fragment were added to the cultures (Fig. 4). As the sera of patients with myasthenia gravis may contain a variety of autoantibodies¹⁶, the specificity of the human antibody preparation was confirmed using sheep anti-*Torpedo* receptor antibodies purified by affinity chromatography¹⁷. The sheep antiserum had previously been shown to react with human skeletal muscle receptors ($45,000 \times 10^{-9}$ mol l⁻¹ against *Torpedo* receptor and 8.7×10^{-9} mol l⁻¹ against human skeletal muscle receptor). This antibody and its F(ab')₂ fragment inhibited the acetylcholine-mediated enhancement of C2 synthesis, whereas neither normal sheep IgG nor its F(ab')₂ fragment did so. The concentration of antibody, specific for the human receptor, required to produce 50% inhibition of the acetylcholine effect was very similar for both human and sheep preparations (2.5×10^{-12} mol l⁻¹ and 1.25×10^{-12} mol l⁻¹ respectively).

Acetylcholine is normally rapidly degraded by cholinesterase. It is probable that the enhanced effect of acetylcholine (Fig. 3) in the presence of neostigmine is due to cholinesterase activity in the cultures. This conclusion is supported by the findings that carbamylcholine, which is resistant to cholinesterase¹⁸, was more potent than acetylcholine (Fig. 1) and that neostigmine did not potentiate the action of this ligand (Fig. 3). Our fetal calf serum contains cholinesterase¹⁹ which is heat stable (117 IU ml⁻¹ before heat-inactivation and 51 IU ml⁻¹ after 2 h

Fig. 3 Effect of cholinergic antagonists and neostigmine on the augmentation of C2 synthesis by *a*, acetylcholine and *b*, carbamylcholine. Conditions for culture and C2 assay were as for Fig. 1. Monocytes were cultured for 14 days in the absence or presence of the cholinergic ligand (10^{-4} mol l^{-1} ; ●). Control cultures and ligand-treated cultures were exposed to atropine (10^{-4} mol l^{-1} ; ○), (+)tubocurarine (10^{-7} mol l^{-1} ; ■) and α -bungarotoxin (10^{-8} mol l^{-1} ; ▼). Each point represents the mean of three experiments performed in duplicate. The horizontal bars represent the standard error of the mean. The enhancement of C2 synthesis by acetylcholine or carbamylcholine is significantly reduced by (+)tubocurarine and α -bungarotoxin ($P < 0.001$ for ligand concentrations of 10^{-4} , 10^{-5} and 10^{-6} mol l^{-1}). Neostigmine (Δ) significantly potentiated the effect of acetylcholine but not that of carbamylcholine ($P < 0.05$). Neither atropine nor the cholinergic receptor antagonists altered C2 synthesis in control cultures (data not shown).

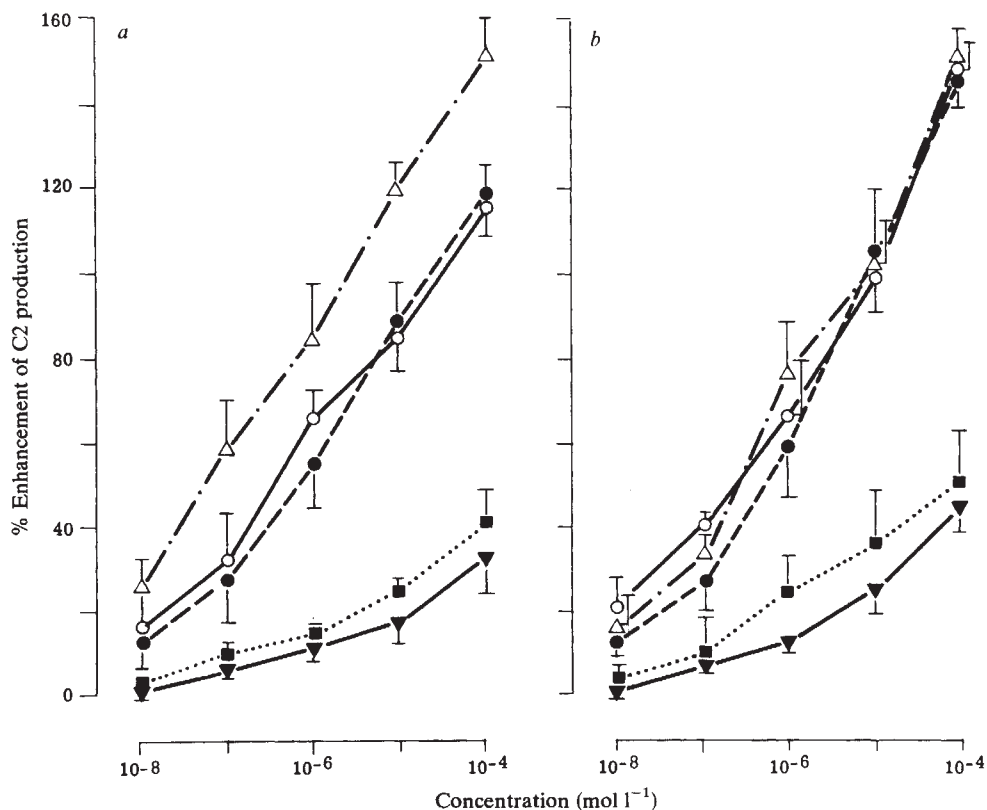
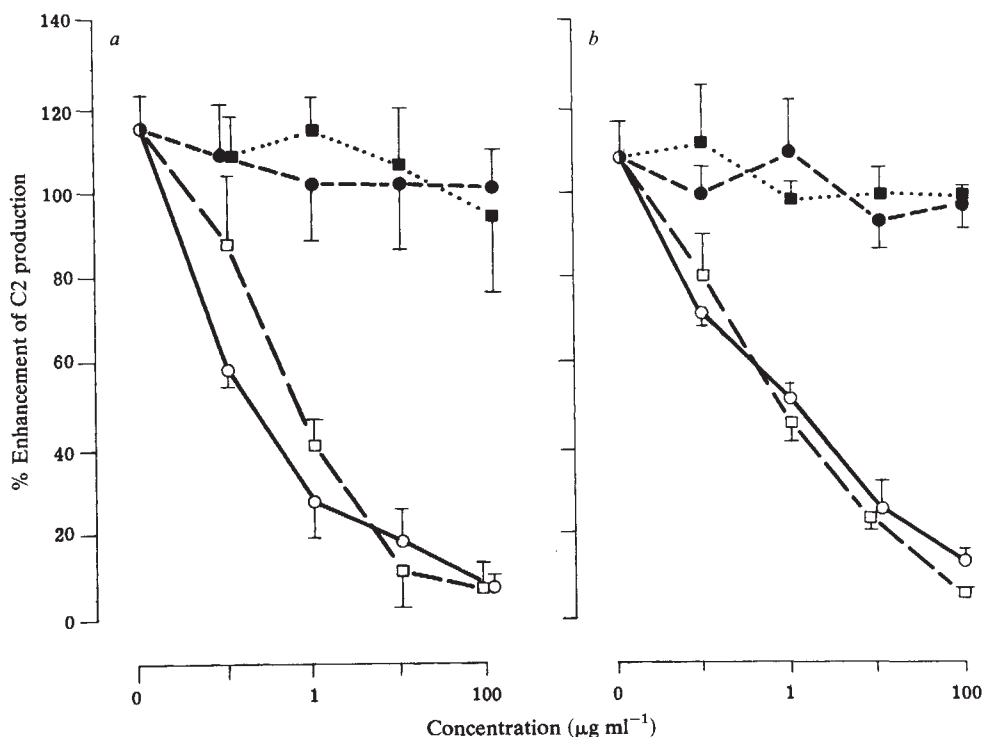


Fig. 4 Antigenic similarity between nicotinic cholinergic receptors on human monocytes and *a*, human skeletal muscle and *b*, electric organ of *Torpedo marmorata*. Monocyte culture conditions and C2 assay were the same as for Fig. 1. Monocytes were cultured in the absence or presence of acetylcholine (10^{-4} mol l^{-1}). *a*, The addition of IgG (○) purified from the serum of a myasthenia gravis patient with circulating acetylcholine receptor antibodies abrogated the effect of acetylcholine, as did the F(ab')₂ fragment¹⁷ (□). The proportion of total IgG reactive with human acetylcholine receptors was 0.2%. IgG prepared from the serum of a normal volunteer (●) and its F(ab')₂ fragment (■) did not significantly alter the acetylcholine effect. The reduction in the acetylcholine-induced enhancement of C2 synthesis produced by IgG anti-receptor antibody and its F(ab')₂ fragment was significant ($P < 0.001$) when their concentrations were ≥ 200 ng ml^{-1} . Normal IgG of IgG anti-receptor antibody or their F(ab')₂ fragments did not significantly affect C2 synthesis in control cultures. *b*, As for *a*, except that immunoadsorbent-purified sheep antibody to the electroplaque of the *Torpedo marmorata* and its F(ab')₂ fragment were used. 0.02% of affinity-purified anti-*Torpedo* receptor antibody was reactive with human skeletal muscle receptors. Normal sheep IgG and its F(ab')₂ fragment served as controls. Symbols as for *a*. The inhibition of the acetylcholine effect produced by the intact antibody or its F(ab')₂ fragment was significant ($P < 0.001$) when their concentrations were ≥ 1 μg ml^{-1} . Anti-receptor antibody, normal sheep IgG and their F(ab')₂ fragments did not alter C2 synthesis in control cultures. Each point represents the mean of three experiments performed in duplicate and the horizontal bars represent the s.e.m. Intact antibodies and their F(ab')₂ fragments had the same effect on carbamylcholine-treated cultures (data not shown).



at 56 °C). We have not excluded the possibility that monocytes contain cholinesterase.

These experiments demonstrate unequivocally that nicotinic cholinergic receptors are present on human monocytes, and that the interaction of these receptors with the ligand increases C2

synthesis. The biological significance of this receptor on monocytes remains to be determined, as do the effect of anti-acetylcholine receptor antibodies and the therapeutic use of anti-cholinesterase on the function of monocytes and serum C2 levels in patients with myasthenia gravis.

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Extracellular phospholipase A₂ mediates inflammatory hyperaemia

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Inflammatory erythema is primarily the gross manifestation of enhanced regional blood flow or hyperaemia. Hyperaemia to areas of injury is thought to modulate the delivery of circulating inflammatory cells to the responding site^{1,2} and in experimental models, it was shown synergistically to enhance the extravasation of plasma proteins and mediators^{3,4}. It has recently been proposed that blood flow to inflammatory sites may be modulated by a novel vasoactive moiety, distinct from prostaglandins and plasminogen activator^{5,6}, which is released by macrophages in response to antigenic or mitogenic stimulation. Intradermal (i.d.) injection of this hyperaemia-inducing activity (HIA) from activated macrophages elicited a sustained hyperaemia in the test rabbit⁷. We have now investigated this vasoactive mediator further. Using a sensitive assay for phospholipase A₂ (PLA₂)⁸, we have identified this enzyme in vasoactive conditioned medium of glycogen-induced peritoneal exudate cells (PEC) of rabbits. In the vasoactive conditioned medium, PLA₂ and HIA cofractionated on gel filtration chromatography, and both the enzyme activity and biological activity were abrogated by treatment with the active site-directed histidine reagent *p*-bromophenacyl bromide (*p*BPB). Intradermal injection of PLA₂ purified from snake venom induced a hyperaemia with kinetics resembling those of HIA. We propose that antigen-induced release of PLA₂ by activated macrophages may mediate the hyperaemia associated with sites of chronic inflammation.

Earlier attempts at the preliminary characterization of HIA released by glycogen-induced PEC of rabbit led to the postulate that PLA₂ was indeed responsible for the observed vasoactivity⁶. In this study, the capacity for PLA₂ (purified from the venom of *Crotalus atrox*) to enhance regional blood flow in rabbits after i.d. injection was quantified by the radiolabelled microsphere method described elsewhere⁷. The kinetics of

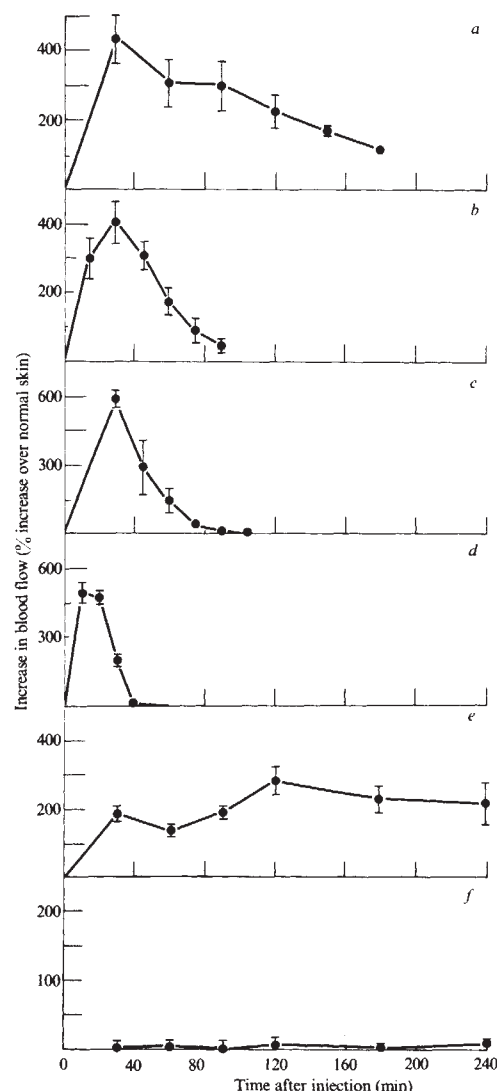


Fig. 1 Kinetics of hyperaemia induced by various vasoactive agents. The putative vasoactive agents were injected i.d. in 0.1-ml aliquots into the dorsal skin of rabbits at various times before the administration of 10- μ m diameter ⁵⁷Co-labelled microspheres (NEN) as described elsewhere⁷. In this way, the blood flow to lesions of various ages was determined in the individual animals. Each point represents the mean per cent increase in blood flow ($n = 18$ sites in three rabbits) $\pm$ s.e.m. The various reagents were made up in phosphate-buffered saline (pH 7.4) to the concentrations indicated: a, arachidonic acid (1,000 μ g ml⁻¹); b, PGD₂ (10 μ g ml⁻¹); c, PGE₂ (10 μ g ml⁻¹); d, PGI₂ (10 μ g ml⁻¹); e, phospholipase A₂ (100 μ g ml⁻¹; from *C. atrox*). f, Injections of physiological saline (0.9%) served as a control.

induction of hyperaemia after i.d. injection are distinct and characteristic for different vasoactive agents^{6,7}. Figure 1 compares the hyperaemia profile of venom PLA₂ with those of arachidonic acid and its stable vasoactive metabolites, prostaglandins (PGs) D₂, E₂ and I₂. Arachidonic acid and the vasoactive prostaglandins showed a rapid onset with peak enhancement of blood flow at 15-30 min after injection, followed by a rapid decay. The vasoactive effect was short lived compared with purified venom PLA₂, which showed a protracted onset of hyperaemia, with peak enhancement of blood flow at 120-180 min after injection, followed by a slow subsidence of hyperaemia. This time course is similar to that described⁶ for the HIA of the conditioned medium of rabbit PEC (PEC-CM). If inflammatory cells release PLA₂ *in situ*, this enzyme might be expected to enhance regional blood flow.

Sterile peritoneal exudates were induced in New Zealand white rabbits (2.5-3 kg) by intraperitoneal (i.p.) injection of

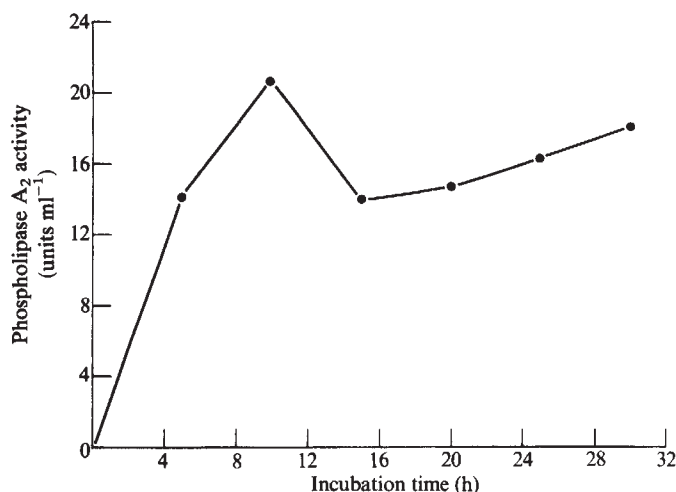


Fig. 2 Kinetics of release of phospholipase A₂ by rabbit PEC. PEC were washed twice and incubated in medium 199 at 37 °C in 5% CO₂ at a concentration of 1×10^7 cells per ml. The cell-free supernatants were collected at 5, 10, 15, 20, 25 and 30 h, and assayed for phospholipase A₂ activity as described previously⁸. The reaction mixture (pH 7.4) consisted of 1.3 ml culture supernatant (or medium 199 control), 100 μ l 10% bovine serum albumin in 0.9% saline and 100 μ l labelled *E. coli* substrate. The reaction was allowed to proceed for 15 min at 37 °C. Results are expressed as the per cent hydrolysis of total radioactivity incorporated. Medium 199 control has been subtracted.

100 ml of 5% glycogen in sterile saline. The PEC were collected and cultured as described previously⁶. The 5-day-old exudates consisted of an average of 60% macrophages, 36% small lymphocytes and <4% neutrophils and erythrocytes as determined by Leishman's staining. Viability of cells, as measured by Trypan blue exclusion, was >85% after 20 h of culture. Replicate cultures were initiated and the PEC-CM assayed at 5-h intervals for the release of PLA₂ activity (Fig. 2), using as the substrate ¹⁴C-oleic acid-labelled *Escherichia coli* which had been autoclaved to inactivate endogenous PLA₂. Details of the modification and of the specificity of this assay are given elsewhere^{8,9}. PLA₂ activity was detected in the culture supernatants, and the kinetics of release of PLA₂ (Fig. 2) were similar to the kinetics of release of HIA from PEC⁶. Release of PLA₂ was essentially complete by 10 h after the cultures had been established. We consistently found diminished activity after 10 h which may have been due to a negative feedback inhibition of activity by reaction products, or perhaps an increased degradation of PLA₂ by other proteolytic enzymes secreted by these cells.

The PEC-CM was fractionated by Sephadex G-50 gel filtration and the eluate assayed for both HIA and PLA₂ activities (Fig. 3). Both the PLA₂ enzyme activity and the HIA biological activity migrated with an approximate molecular weight of 10,000–13,000. This relatively high molecular weight (observed in dissociating buffer) further distinguished HIA from the low-molecular weight prostanoid fatty acids and vasoactive peptides such as kinins, angiotensins and vasoactive intestinal peptide.

pBPB is an active site-directed histidine reagent that inactivates PLA₂s from snake venom¹⁰, horse¹¹, sheep¹² and pig pancreas¹³. Briefly, PEC-CM was dialysed against 50 mM Tris, 50 mM EDTA buffer pH 7.5 and 1.15 ml of the medium was reacted with 60 μ l of 2 mM pBPB in acetone for 30 min at 22 °C. Acetone alone was added to controls. The reaction mixtures were dialysed exhaustively against 50 mM Tris, 25 mM CaCl₂ buffer pH 7.5. Samples were assayed for HIA and PLA₂ activities. The reaction mixture for the PLA₂ assay consisted of 200 μ l PEC-CM, 1.0 ml 50 mM Tris, 25 mM CaCl₂ buffer pH 7.5, 200 μ l 10% bovine serum albumin and 100 μ l labelled *E. coli* substrate. The reaction was allowed to proceed for 15 min at 37 °C. Treatment of the active column fractions (see Fig. 3) with

pBPB resulted in a loss of PLA₂ enzyme activity with a concomitant loss of HIA activity.

Table 1 compares HIA and PLA₂ activities on the basis of these criteria. The similarity of the respective activities strongly suggests an identity of PLA₂ with HIA; various observations in the literature support this contention. In rabbits, the intra-tracheal administration of cobra venom factor contaminated with PLA₂ evoked a massive inflammatory response¹⁴. The pro-inflammatory potential of this preparation was completely abolished by inactivation of PLA₂ with pBPB. The extracellular PLA₂ may exert its effects by the generation of biologically active reaction products such as the prostaglandin family of arachidonic acid derivatives, cytotoxic free fatty acids¹⁵ and the highly pro-inflammatory lysophosphatidic acids¹⁶. These lysoderivatives are also potent platelet aggregators¹⁷, and exert systemic vasoactive effects¹⁸. It is interesting to note that the non-steroidal inhibitors of PLA₂, specifically, mepacrine, pBPB and CB874, all showed anti-inflammatory activity as assessed by their effect on carrageenin-induced rat paw oedema¹⁹.

Although evidence implicates the activated macrophage as the source of the HIA⁶ and PLA₂⁸, the possible contributions by other cell types remain to be resolved. However, the extracellular release of PLA₂ is not without precedent. PLA₂ activity has been detected in the cell-free supernatant fraction of sterile peritoneal exudates produced in rabbits²⁰, as well as in lymph plasma draining inflamed sites²¹ in sheep. Regardless of whether the mechanism involves active secretion or passive release, the

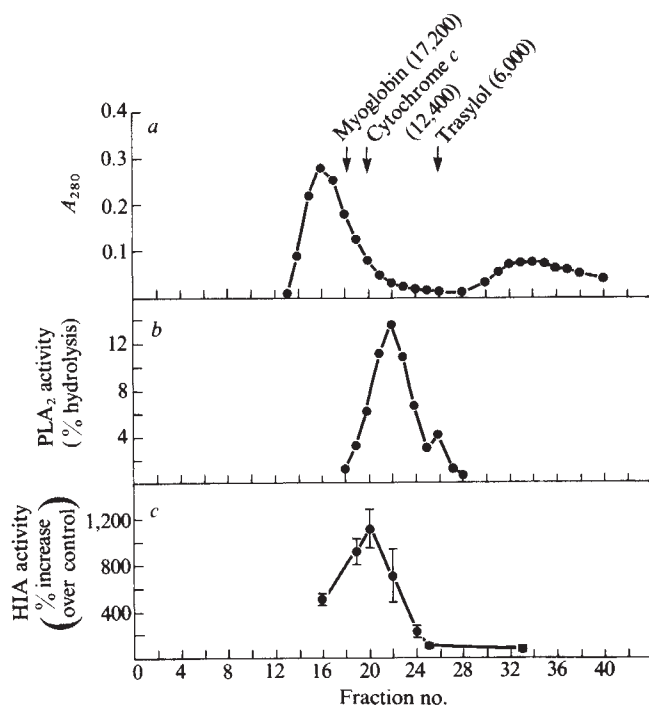


Fig. 3 Gel filtration of PEC-CM, using Sephadex G-50. PEC were incubated for 24 h in medium 199 as described in Fig. 2 legend. The conditioned medium was centrifuged at 500g for 10 min to remove cells and debris. The supernatant was lyophilized and reconstituted in one-tenth the original volume and exhaustively dialysed, using Spectrapor tubing 3 (Spectrum Medical Industries, MW cut-off 3,500), against 50 mM Tris, 25 mM CaCl₂, 1.5 M NaCl pH 7.5. An aliquot of the dialysed sample (1.0 ml) was applied to a 1 × 55 cm Sephadex G-50 column equilibrated and eluted with 50 mM Tris, 25 mM CaCl₂, 1.5 M NaCl pH 7.5. Fractions of 1.1 ml were collected and analysed for a, protein content; b, phospholipase A₂ activity; and c, hyperaemia-inducing activity. The column was calibrated with protein markers of known molecular weight; their elution positions are indicated in panel a. The HIA and PLA₂ activities were assayed as described in legends to Figs 1 and 2 respectively.

Table 1 Comparison of HIA and PLA₂ activity

Criteria for comparison	HIA	PLA ₂
Hyperaemia on intradermal injection	Yes	Yes
Kinetics of induction of hyperaemia in rabbit skin	2-h peak	2-h peak
Kinetics of release <i>in vitro</i> ⁶	Maximum at 10 h	Maximum at 10 h
Inhibition of release <i>in vitro</i> ²⁷ by:		
Indomethacin	No	No
Dexamethasone	Yes	Yes
Molecular weight	10,000–13,000	10,000–13,000
Inactivation by pPBP	Yes	Yes

liberation of PLA₂ into the interstitium may represent a mode of amplification of PG generation by increasing the available phospholipid substrate of the lipid membranes of surrounding cells. The enhanced formation of PG by the injection of exogenous PLA₂ is well documented²².

The observed release of PLA₂ in response to inflammatory stimuli and the hyperaemia-inducing effect of exogenous PLA₂ suggest a common mechanism of action of anti-inflammatory drugs such as mepacrine and the corticosteroids. Steroidal anti-inflammatory drugs inhibit PLA₂ activity^{23,24} and also abolish hyperaemia and other signs of the inflammatory response. In fact, there is a direct correlation between the anti-inflammatory efficacy of corticosteroids and their ability to inhibit PLA₂ activity²⁵. Mepacrine, a non-steroidal inhibitor of PLA₂²⁵, is effective in the treatment of rheumatoid arthritis and systemic lupus erythematosus.

The data from these experiments and others from the literature support the contention that PLA₂ released from inflammatory cells has a central role in the mediation of hyperaemia and may therefore modulate other parameters of the inflammatory response. The anti-inflammatory efficacy of certain steroidal and non-steroidal drugs may partially reside in their ability to inactivate extracellular PLA₂.

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Multiple immunoglobulin switch region homologies outside the heavy chain constant region locus

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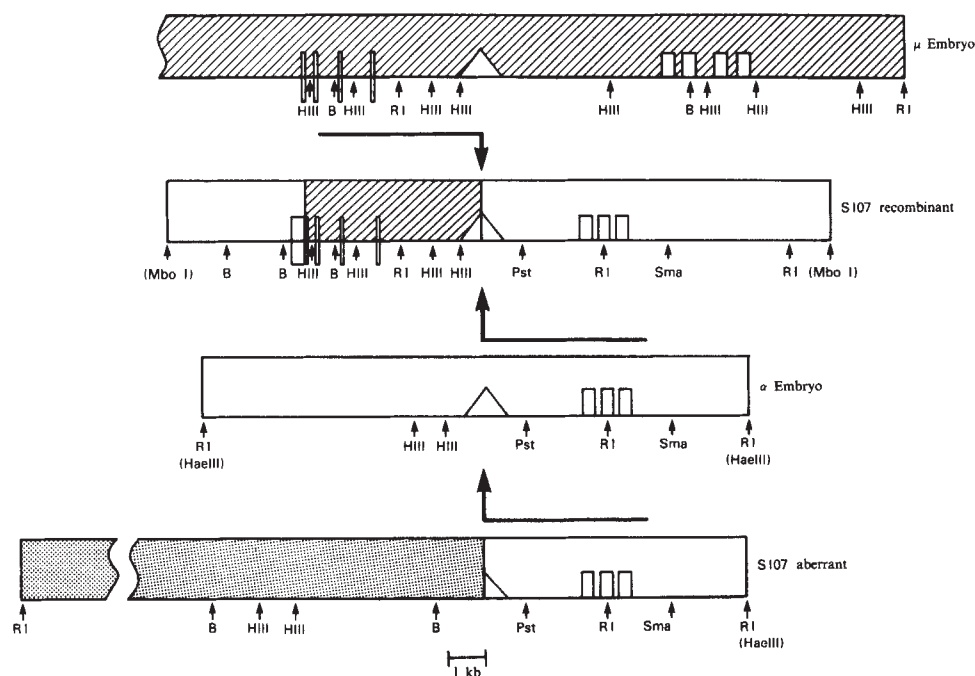
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Somatic rearrangement of gene segments enables higher organisms to generate additional genetic diversity from an essentially limited and otherwise linear genome. The only known example of this powerful mechanism in vertebrates is the immune system, in which active genes are somatically assembled from distant pieces of DNA^{1–3}. This process operates both in V–J (light chain) or V–D–J (heavy chain) joining, to form novel variable region genes from widely separated coding segments^{4–9}, and in heavy chain class switching, whereby assembled variable region genes are switched from one heavy chain gene to another^{10–14}. The latter process correlates with the sequential appearance of the same V region in association with different heavy chains during B-cell development^{15–22}. Conserved sequences adjacent to these genes may be required for the two processes^{4–6,23–28}. In the case of the heavy chain class switch from the μ to the α gene, such sequences involve 2–3-kilobase (kb) segments characterized by tandem pentanucleotide and higher-order repeats^{24–26,28}. We have previously shown that this region has been selectively conserved in both mouse and man and therefore changed little during the 70 Myr since these species have diverged²⁸. Using the switch segment as a hybridization probe, we now show that approximately 15 switch-like regions are present in germ-line DNA and that only one is deleted during the μ – α switch that has occurred in the IgA-producing cell line, S107. As the heavy chain constant region locus from μ through ϵ is deleted in this cell line (including all the other known heavy chain genes), we conclude that the remaining switch-like signals lie outside this region.

Gene cloning techniques were used to characterize the heavy chain loci of the IgA-producing plasmacytoma line S107 (ref. 29). Two allelic copies of the heavy chain gene have been rearranged in this cell so as to involve the α gene (see map of the S107 recombinant and S107 'aberrant' in Fig. 1, and the rearranged 5- and 18-kb *EcoRI* fragments that hybridize to an α probe in Fig. 2, lanes α). One of the rearranged genes corresponds to the α chain produced by the S107 cell, while the other is an aberrant recombinant in which a fragment of DNA, not obviously related to the immunoglobulin system, has been joined to the switch site close to the α coding region. Both rearrangements have resulted in loss of the genetic sequences lying between the μ and the α switch segments (see maps, Figs 1 and 2, and description below). Deletion of μ constant regions has been noted in experiments designed to establish the nature of the switching mechanism¹¹. Our data are consistent with this result except that we can detect rearranged V regions in S107 DNA (data not shown) and the deletion of the γ and ϵ genes as well (see below).

Previous studies using a DNA fragment containing the μ switch region have shown that it cross-hybridizes with several restriction fragments²⁶. These are confirmed by the analysis shown in Fig. 2. The data presented here further show that only one of the more than 15 cross-hybridizing fragments detected with the μ switch probe disappears in the S107 cell. The lost fragment, a 12-kb band, is replaced by a new 5-kb band, as

Fig. 1 Maps of germ-line mouse μ and α and S107 rearranged α genes, constructed from cloned DNA fragments from partial restriction endonuclease digests of total mouse 12-day embryo (J. G. Seidman, personal communication) or S107 DNA using cDNA and genomic probes. The generation of the libraries³⁰, isolation of the clones with the phage λ vectors CH4A³¹ and λ CH28 (F. Blattner, personal communication), the phage packaging system³² and an *in situ* hybridization technique³³ have been described elsewhere. Open bars indicate the coding domains of the α and μ constant regions. Elongated rectangles indicate the mouse heavy chain J segments and the V-D-J segment of the S107 recombinant. The triangle covers an area of switch region homology between the μ and α genes. The μ and μ 'footprint' regions are indicated by cross-hatching; the 'foreign' DNA of the S107 'aberrant' gene by stippling. A line shows the presumed recombination event between μ and α that resulted in the formation of the S107 recombinants. Relevant restriction sites for each gene fragment are shown (RI, *EcoRI*; B, *Bam*HI; HIII, *Hind*III).

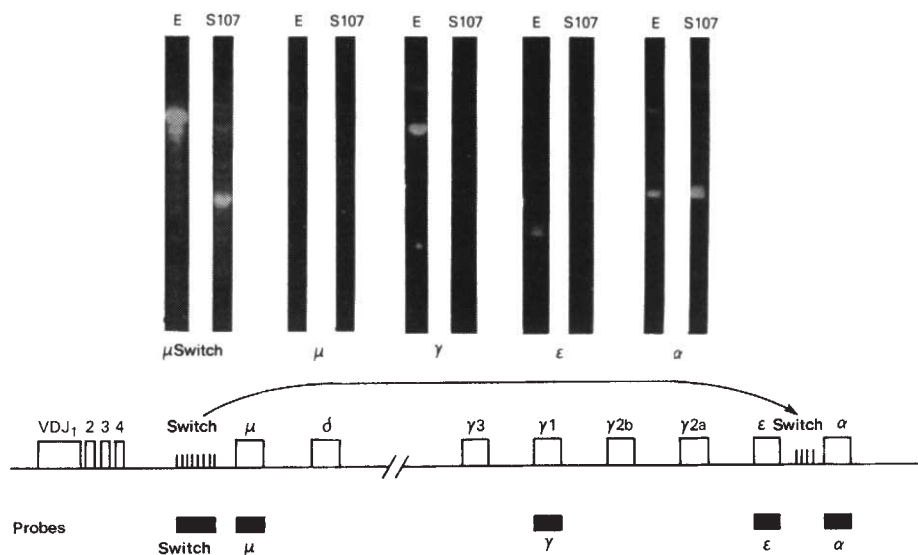


shown in Fig. 2, lanes ' μ switch'. That the region between the μ and α genes is deleted from the S107 genome is shown by the disappearance of the μ , γ and ϵ fragments from the DNA (Fig. 2, lanes μ , γ and ϵ). In our hybridization conditions (see Fig. 2 legend), the μ switch region probe hybridizes to the α , but not to the γ or ϵ switch regions²⁸. Because of this and the deletion of the DNA segment between μ and α , we conclude that there are multiple segments of DNA in the mouse genome that are homologous to the μ switch region, almost all of which are

encoded beyond the confines of the constant region locus as defined 5' by the μ switch region and 3' by the α constant region.

The physiological significance of multiple switch-like sequences positioned at some unknown distance from the immunoglobulin heavy chain constant region locus cannot yet be evaluated. These sequences could represent pseudocopies of the μ switch region; that is, sequences of no functional value that arose by gene duplication, that have been scattered along the chromosome on which heavy chain genes are located or even

Fig. 2 Hybridization of mouse embryo and S107 cell line DNA to probes from the immunoglobulin heavy chain locus. Twelve-day total mouse embryo and S107 cell line DNA was digested with *Eco*RI, electrophoresed on 0.75% agarose, transferred to nitrocellulose by the method of Southern³⁴ and hybridized with the ³²P-labelled probes indicated on the schematic map (not drawn to scale). The μ switch probe was a 1.5-kb *Hha* to *Hha* fragment located 2 kb 5' from the μ coding region and was found by nucleotide sequencing to contain the characteristic sequence repeats previously described in mouse switch regions (refs 23-27 and J.V.R., unpublished results). The constant region probes were (1) a 0.8-kb *Hha* to *Hha* μ probe; (2) a 2.5-kb *Sac* to *Sac* γ 1 probe; (3) a 4.5-kb *Bam* to *Bam* ϵ probe; and (4) a 3.2-kb *Sma* to *Xba* α probe. Low-stringency hybridization conditions ($6\times$ SSC, 70°C) and high-stringency washes ($0.1\times$ SSC, 0.1% SDS, 52°C) were used. The resulting autoradiograms shown are a compilation of several experiments; no attempt has been made to align comparable fragment sizes across all the lanes, only within each comparison of embryo (E) and S107 cell line DNA. The autoradiograms demonstrate that sequences identified in embryo DNA by the constant region probes for μ , γ 1 and ϵ have been deleted in S107. The α probe identifies two *Eco*RI fragments in embryonic DNA because the α gene contains an *Eco*RI site in the second coding block (see Fig. 1). The smaller (~4.2 kb) 3' fragment remains unchanged in S107 DNA as expected, because the μ - α switching events occur 5' to this fragment. The larger α -containing (~10 kb) 5' fragment in embryo DNA is replaced in S107 by fragments of ~18 kb and ~5 kb, reflecting a different rearrangement on each of the two homologous chromosomes bearing heavy chain genes. Clones corresponding to both of these fragments have been obtained (I.R.K. and S.P.K., unpublished results) and characterized by nucleotide sequencing; they correspond, respectively, to the 'S107 aberrant' and 'S107 recombinant' genes shown in Fig. 1. The μ switch probe identifies ~15 hybridizing bands in both embryo and S107 DNA. The strongest of these in embryonic DNA corresponds in size to the ~12 kb embryonic *Eco*RI fragment containing both the μ switch and coding regions (see map, Fig. 1), the fragment from which the probe was derived. This band is replaced in S107 DNA by an intense ~5-kb band corresponding to the functional recombinant gene. Taken together, these blots support the conclusion that on both chromosomes in S107, rearrangements occurred which deleted the entire region between the μ switch and the α switch (indicated by the long arrow). The other 15 or so *Eco*RI fragments which hybridize to the μ switch probe in S107 must therefore lie outside this locus. The arrow indicates the rearrangement occurring in the μ - α switch as is presumed to have occurred in the formation of the S107 heavy chain gene. Gene linkage has been established for the entire region except for the area between δ and 5' of γ 3 (refs 35, 36). The figure is a compilation of data from several experiments; no attempt has been made to indicate comparative fragment size by alignment across all the lanes.



transposed to other chromosomes where they undergo evolutionary drift. On the other hand, the fact that analogous sequences are involved in the somatic recombination of antibody genes makes these multiple switch-like segments attractive candidates for a recombinational function as yet unidentified in the immune system or in some other system of somatically recombining genes.

Note that in the 'aberrantly' recombined α gene present in S107 (Figs 1, 2), a segment of DNA has been joined directly to the α switch region. When a probe is prepared from this segment, it detects a single *Eco*RI fragment of DNA in embryonic DNA (its germ-line configuration) and the same germ-line fragment and itself in S107 DNA (Fig. 3, lanes E and S107). The joined fragment obviously also lies outside the μ - α switch region borders of the heavy chain locus in germ-line DNA. It does not hybridize to S107 V or J region probes (data not shown). As this segment has been joined directly to the switch region, it may have been joined to the α switch locus by a recombination event facilitated by a switch-like complementary sequence. We have not yet been able unequivocally to identify one of the μ switch fragments as that corresponding to the embryonic configuration of this joined segment. We also do not know the functional significance, if any, of this segment. However, this DNA segment rearranges in other myeloma cell lines. Figure 3 shows an *Eco*RI digest of DNA derived from myeloma cell lines S176 (IgA- λ) and TEPC15 (IgA-K) as well as from S107 and mouse embryo; germ-line and rearranged bands appear in each. We have seen this fragment rearranged in five of six different IgM and IgA lines of varying idotype tested and therefore suspect that it may be relevant, in some as yet unknown way, to heavy chain formation.

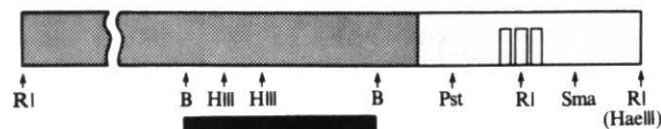
The switch segments within the immunoglobulin heavy chain locus allow a powerful mechanism to operate so as to create new genetic possibilities within the humoral immune system. The finding of multiple switch-like segments outside the immunoglobulin locus will prompt further investigation into the behaviour of these segments in other gene systems.

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E TEPC S S
15 176 10



Fig. 3 Identification of rearrangeable DNA fragments in mouse embryo and three myeloma cell line DNAs. A map of an aberrantly rearranged α immunoglobulin gene segment derived from the S107 cell line is shown and labelled as in Fig. 1. The filled bar beneath the map represents a probe used to detect the rearranged fragment. *Eco*RI-digested DNAs from total mouse embryo and myeloma cell lines TEPC15, S176 and S107 were prepared and blotted as described in Fig. 2 legend. The 5.5-kb 32 P-labelled probe was prepared following *Bam*HI digestion and subsequent subcloning. There is a single large (>22 kb) *Eco*RI fragment in embryonic DNA. Each myeloma DNA retains this fragment and also demonstrates a second rearranged fragment. The fragment identified in the S107 DNA corresponds to the ~18-kb *Eco*RI fragment from which the probe was derived.



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Structure of binding sites for heterotropic effectors in fish haemoglobins

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Haemoglobin binds oxygen cooperatively. In mammalian and bird haemoglobins the oxygen affinity is modulated by hydrogen and chloride ions, organic phosphates and CO₂ (ref. 1), and the amino acid residues responsible for these so-called heterotropic effects have been identified^{2,3}. In some fish haemoglobins these heterotropic effects are modified to adapt the fish to their specific habitats⁴. We have determined the functional properties and amino acid sequences of the haemoglobins in trout blood and in the Amazonian fish *Pterygoplichthys pardalis* (Pt HbI), which is a facultative air breather⁵⁻⁹. Trout HbI binds oxygen cooperatively, but shows no Bohr effect over the pH range 5-9 and no organic phosphate effect¹⁰. Pt HbI also binds oxygen cooperatively; its Bohr effect is small and reversed and its oxygen affinity is lowered by organic phosphates below pH 7.0-7.5 (ref. 9). Both haemoglobins represent substantial fractions of the total pigments in the red cells. We have now analysed the amino acid sequences of these two proteins to discover whether the particular residues thought to be responsible for the Bohr effect and organic phosphate binding in mammalian haemoglobins have been replaced by others in fish haemoglobins^{6,7,11}.

Table 1 shows our results, which are consistent with the attribution of the major part of the alkaline Bohr effect in mammalian haemoglobins to valine 1 α and histidine 146 β . On the other hand, they are not consistent with the suggestion that ~25% of the alkaline Bohr effect in haemoglobin A (HbA) is due to histidine 122 α , as this is also present in trout HbI¹².

Figure 1 shows the residues in the crevice between the two β -chains, which was originally shown by Arnone¹³ to be the

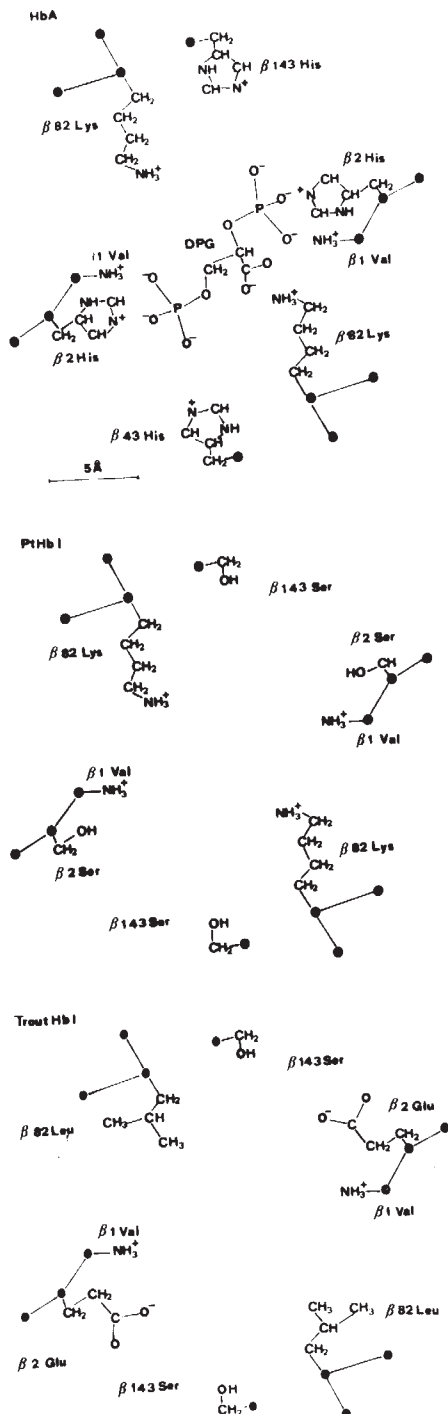


Fig. 1 Schematic drawing of the binding site for organic phosphates in HbA, as indicated by Arnone¹³ (top); analogous schemes for Pt HbI (middle) and trout HbI (bottom) are also shown, to indicate the profound and characteristic changes in the arrangement of charges which have occurred in the central crevice of these two fish haemoglobins.

Table 1 Ligand-linked groups in human HbA, trout HbI and *Pterygoplichthys* HbI

	HbA	Linked with DPG	Cl ⁻	Trout HbI	Pt HbI
α chain					
Val 1	—	+	+	Ac-Ser	Ac-Ser
His 122	—	—	?	His	?
β chain					
Val 1	+	+	±	Val	Val
His 2	+	+	±	Glu	Ser
His 143	+	+	±	Ser	Ser
His 146	—	—	—	Phe	Phe

All the residues involved in the Bohr effect either directly or through linkage with DPG and/or Cl⁻ binding are indicated.

diphosphoglycerate (DPG) binding site in deoxyHbA. Subsequent experiments have confirmed this³. Figure 1 also shows the corresponding residues in the two haemoglobin components from trout and *Pterygoplichthys*. In trout HbI, the amino acid residues involved in the binding of DPG are all substituted by unfavourable side chains; in particular, Lys 82 β is substituted by Leu and His 2 β by Glu. These substitutions, which abolish the cluster of positive charges around the dyad axis of the molecule and instead insert two negative charges (Glu 2 β), account for the lack of binding of organic phosphates by trout deoxyHbI and oxyHbI. We have recently discovered that the binding of sodium ions is O₂ linked in trout HbI¹⁴. On the other hand, in Pt HbI, Lys 82 β , which is a critical residue in the binding of DPG, is conserved and His 2 β is substituted by Ser. Therefore, the organic phosphate effect is still observed in Pt HbI, although the affinity constant is smaller than in HbA (ref. 9 and unpublished results).

The close agreement of these structural data with the information available for HbA is remarkable, and strongly supports the conclusions for the mammalian protein^{3,4}. However, it is not consistent with the attribution of the alkaline Bohr effect to the sum of small contributions from many residues¹⁵. The physiological significance of these differences in heterotropic effects in the various organisms, in relation to their O₂ demands in different environments, is unknown. Although cooperative O₂ binding is a characteristic feature of all tetrameric haemoglobins, the heterotropic effects are often variable and/or absent in haemoglobins from lower vertebrates. Perhaps the fine tuning of O₂ release, operated through heterotropic phenomena, evolved from a 'core' of functional and structural features underlying the homotropic effects, possibly by a gene-duplication mechanism¹⁶.

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Demethylation of CpG sites in DNA of early rabbit trophoblast

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The function of DNA methylation in eukaryotes is unknown. The introduction of methyl groups into the major groove of B-form DNA has been shown to affect DNA-protein interactions in prokaryotes¹, and it is probable that they perform analogous functions in eukaryotic DNA. Thus, there is considerable interest in the possibility that DNA methylation may be involved in regulating DNA transcription and in establishing stable patterns of genetic expression in higher organisms^{1,2}. Restriction enzyme studies have shown that methylation of eukaryotic DNA is not random; methylation patterns are tissue specific when individual genetic loci are examined³⁻⁵. On the other hand, it is also apparent that these tissue-specific patterns tend to be rather subtle and to occur within overall levels of methylation that are relatively constant in all tissues of a given organism⁴. For example, no significant change in total DNA methylation has been detected during embryogenesis in either the sea urchin^{6,7} or the mouse⁸, even though rapid cellular differentiation is occurring. We present evidence here, however, for extensive changes in methylation accompanying early differentiation in the rabbit embryo. Whereas the DNA of the early cleaving embryo is highly methylated, as is that of the later embryoblast, the terminally differentiated trophoblast⁹ is demethylated by about 35%.

Almost all methylation in mammalian DNA occurs as methylcytosine (MeC) in the sequence 5'-CpG (ref. 10). As pointed out by Bird and Southern¹¹, the bacterial endonucleases *HpaII* and *MspI* are especially useful in probing CpG methylation. Both enzymes cut DNA at the sequence 5'-CCGG; *HpaII* will not cut if the internal cytosine is methylated whereas *MspI* will¹². Thus differences between the observed fragment sizes resulting from complete digestion with *HpaII* and *MspI* can be ascribed solely to 5'-CMeCGG. From published values for base composition and nearest-neighbour frequencies¹³, we calculate a value of 2.2 kilobase pairs (kbp) for the weight-average length of rabbit DNA fragments expected after digestion with *MspI*¹⁴. As shown in Fig. 1a, when embryonic DNA is radiolabelled with [$Me-^3H$]thymidine, digested to completion with *MspI* and electrophoresed in 1.6% neutral agarose, the resulting fragments are distributed over a broad size range. The observed weight-average length—the point in the gel at which equal amounts of radioactivity are recovered in both larger and smaller fragments—corresponds to 2.4 ± 0.2 kbp. We therefore conclude that essentially all 5'-CCGG sites are accessible to *MspI*.

As shown in Fig. 1b, few of these sites in the DNA of the early cleaving embryo are cut by *HpaII*. The weight-average length of these fragments is 7.2 ± 0.4 kbp. As the weight-average length is twice the number-average length in random polymers¹⁵, and the number of fragments produced from a given length of DNA is one greater than the number of enzymatic cuts, we calculate that *HpaII* cuts only 4.6 of 15.7 possible 5'-CCGG sites in this DNA preparation of ~20 kbp. Thus, 11.1 of the 15.7 sites (71%) seem to be methylated in the DNA of the early cleaving embryo.

By the third day of development, the morula resulting from cleavage divisions begins to be transformed into an early blastocyst in which trophoblast and embryoblast (inner cell mass) cells are visibly differentiating. The DNA in these early blastocysts was found to be more extensively cut by *HpaII* (Fig. 1c). The weight-average length of these fragments has decreased to 4.4 ± 0.1 kbp which, by the calculation indicated above, corresponds to 48% methylation at 5'-CCGG sites. This reduced level of methylation is also apparent in the DNA of the late blastocyst at 6–7 days of development (Fig. 1d).

The large size of the late rabbit blastocyst makes it possible to separate embryoblast and trophoblast cell populations manually so that their DNAs can be analysed in isolation. As shown in Fig. 2, the pattern of *HpaII* digestion obtained with total blastocyst DNA (Fig. 1d) reflects that of the trophoblast, which comprises about 95% of the cell population at this stage¹⁶. The *HpaII* digestion pattern of embryoblast DNA, on the other hand, is very similar to that of the early cleaving embryo. The contrast between trophoblast and embryoblast DNAs is also seen when other CpG enzymes are used—*HhaI* (recognition sequence 5'-GCGC) and *SalI* (5'-GTCGAC)—also shown in Fig. 2. It is known⁴ that both enzymes are prevented from cutting DNA by MeCpG, but as isoschizomers of these enzymes are not available, their failure to cut embryoblast DNA as effectively as

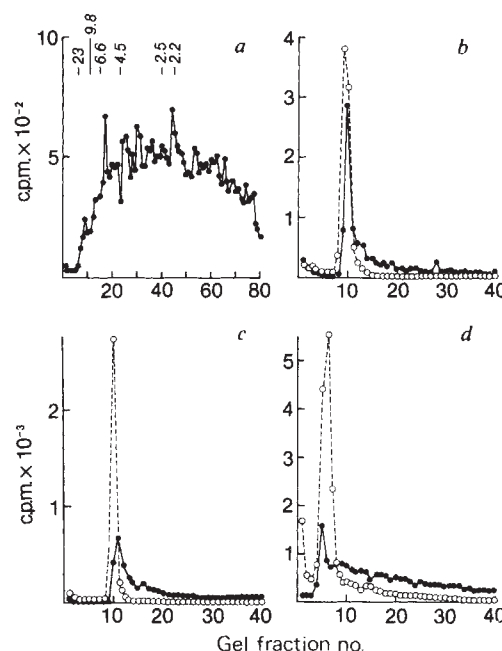


Fig. 1 Cytosine methylation in the sequence 5'-CCGG in the DNA of the pre-implantation rabbit embryo. Embryos from New Zealand White rabbits were radiolabelled by incubation *in vitro* in modified F-12 solution²² containing 5% rabbit serum and [$Me-^3H$]thymidine (NEN; 50–60 Ci mmol⁻¹) at 2.5–5.0 μ Ci ml⁻¹ for 4–20 h. The zona pellucidas were then removed and the embryos digested overnight at room temperature in 0.1–1.0 ml 100 mM NaCl, 10 mM EDTA, 0.6% SDS containing proteinase K at 100 μ g ml⁻¹. The digests were then extracted with an equal volume of phenol/chloroform (1:1) followed by a second extraction with an equal volume of chloroform/isoamyl alcohol (24:1). Nucleic acids were precipitated from the final aqueous phase in 0.2 M NaCl by the addition of 2.5 volumes of 95% ethanol. Precipitates were collected by centrifugation, dried under vacuum and resuspended in the appropriate buffer for endonuclease digestion. Control samples were mock-digested in *HpaII* buffer. Phage λ DNA (1 μ g) was added to each reaction mixture to monitor the completion of digestion. At least a fourfold excess of enzyme was used and the mixtures were incubated at 37 °C for 1 h. The digests were then applied to 5 $\times$ 110 mm tube gels of 1.6% agarose. Electrophoresis was carried out in E buffer (40 mM Tris, 20 mM Na acetate, 2 mM EDTA pH 7.8) at 1 mA per gel for 16 h. Gels were stained with ethidium bromide and phage λ DNA fragments identified under UV light. The gels were then fractionated into 1 mm slices using a Mickel gel slicer. The gel slices were solubilized in 150 μ l 1 M HCl in a boiling water bath for 20 min, cooled to room temperature and mixed with 3.5 ml BetaGel (WestChem Products). Radioactivity in gel fractions was determined to <5% s.e. in a Beckman LS-230 liquid scintillation counter. *HindIII* digests of phage λ DNA were electrophoresed in parallel gels to provide fragment size standards. The radioactivity profiles shown were verified by duplicate or triplicate assays of separate embryonic samples. **a**, *MspI* digest of DNA from the late 6-day blastocyst; the position of phage λ DNA fragments after *HindIII* digestion is shown across the top. **b**, DNA from cleaving embryos radiolabelled from the 2-cell to the 8-cell stage (20 h). **c**, DNA from embryos radiolabelled for 20 h at the morula-early blastocyst transition. **d**, DNA from late 6-day blastocysts radiolabelled for 4 h. Dotted lines in **b**, **c** and **d** are the mock-digested control sample, and solid lines represent the radioactivity in the *HpaII* digest. Approximately equal c.p.m. were applied to each of the paired gels at each embryonic stage. For comparative purposes, the total c.p.m. recovered from over 100 slices of each gel were determined and equalized for each pair, although only the 40 fractions from the origin are shown in the figure. The native DNA in all preparations migrated with an apparent length of 20–25 kbp.

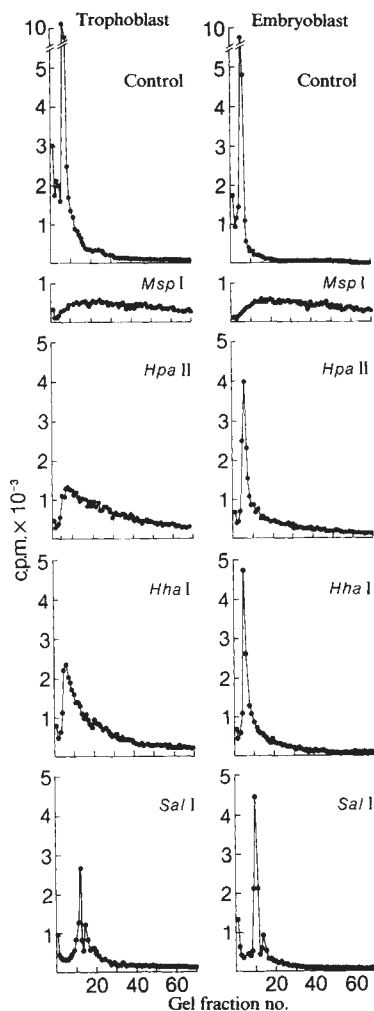


Fig. 2 Restriction endonuclease digests of ^3H -DNA from trophoblast and embryoblast cells of the late 6-day rabbit blastocyst. Intact blastocysts were radiolabelled *in vitro* with [$\text{Me-}^3\text{H}$]thymidine at $5 \mu\text{Ci ml}^{-1}$ for 4 h. The embryoblast area was then excised from each blastocyst; the incorporated radioactivity in these fragments averaged 4.5–5.5% of the total. DNA was extracted from the pooled trophoblast and embryoblast fragments, digested with the indicated endonuclease and electrophoresed as described in Fig. 1 legend, except that 1.2% agarose gels were used for the *SalI* digests. Approximately 75,000 c.p.m. were applied to each gel. To allow direct comparisons between trophoblast and embryoblast with each enzymatic digestion, the c.p.m. totals recovered from each pair of gels were normalized. The profiles shown are the result of duplicate or triplicate analyses.

trophoblast DNA is only probably, rather than certainly, due to differences in cytosine methylation.

Singer *et al.*⁸ estimate that ~50% of the 5'-CCGG sites in DNA from adult rabbit tissues are methylated, a value almost identical to the one we derive for the early trophoblast. Thus although the methods used to arrive at these values differ, it may be that the trophoblast in the rabbit is the first cell type to acquire the 'adult' level of methylation, perhaps by virtue of its status as a terminally differentiating cell, and that the extensive methylation of embryoblast DNA is characteristic of developmentally pluripotent cells. It is evident that this divergence in methylation patterns begins as early as the fourth day of embryogenesis, some 9–10 cell divisions after zygote formation¹⁶ (Fig. 1c). The biological evidence allows the conclusion that at least some of the blastomeres remain totipotent¹⁷ through the 8-cell stage of rabbit embryogenesis. Although it is conceivable that the minor amount of hypomethylated DNA detectable in the cleaving embryo (Fig. 1b) becomes preferentially segregated into the blastomere(s) destined to become trophoblast, we consider it more likely that DNA is uniformly methylated in all blastomeres at least to the 8-cell stage and that demethylation of about 1/3 of the CpG sites accompanies

trophoblast differentiation. Note also that rabbit sperm DNA is found to be extensively methylated at 5'-CCGG sites¹².

Overall CpG methylation in radiolabelled DNA of the mouse embryo is reported to remain essentially constant during this same early period; 5'-CCGG sites are about 75% methylated in the mouse embryo as well as in the adult⁸. It seems probable that the reported analyses of radiolabelled total mouse blastocyst DNA reflect primarily methylation of embryoblast DNA rather than the trophoblast pattern we find in the rabbit. The mouse blastocyst contains not only a higher proportion of embryoblast cells than does the rabbit⁹, but in addition these embryoblast cells are known to be more active in DNA synthesis than the trophoblast¹⁸. It will be of great interest, although considerably more difficult, to isolate mouse and other mammalian trophoblast cells for analysis of their DNA methylation in comparison with embryoblast and adult cell patterns.

Where attempts have been made to relate DNA methylation to transcriptional activity^{3–6}, a general but by no means universal finding is that unmethylated sites in DNA are more actively transcribed. Exceptions to this rule include the highly methylated sequences encoding ribosomal¹⁹ and 5S RNA²⁰ in *Xenopus* DNA which are actively transcribed, as well as the unmethylated $\gamma\delta\beta$ -globin locus in human placental DNA which is not transcribed⁴. Our results further emphasize the complexity of the relationship between DNA methylation and transcription. Previous autoradiographical studies in this laboratory have indicated that the highly methylated rabbit embryoblast DNA is transcribed more extensively than the hypomethylated trophoblast DNA²¹.

In the system we describe here, demethylation of DNA can be more obviously related to loss of developmental potential. The asymmetric cell division(s) that must occur in this initial differentiative event to segregate the unipotent trophoblast from the pluripotent embryoblast is formally analogous to the stem cell phenomenon discussed by Holliday and Pugh², although demethylation of the DNA in the terminally differentiating cell is occurring rather than the *de novo* methylation proposed in their model. Nonetheless, our findings indicate that mechanisms do exist in the cells of higher organisms to bring about rapid and extensive alterations in DNA methylation, and that this form of DNA modification can parallel developmental events.

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Note added in proof: By radiolabelling late rabbit blastocysts simultaneously with [$\text{Me-}^{14}\text{C}$]thymidine and [$\text{Me-}^3\text{H}$]L-methionine, we have recently found that the $^3\text{H}/^{14}\text{C}$ ratio in trophoblast DNA is 64% of the value obtained in embryoblast DNA, a finding consistent with the enzymatic data presented here.

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BOOK REVIEWS

Learning beyond stimulus-response

Stuart Sutherland

SINCE the time of Pavlov, most theories of animal learning have been distorted by the idea that all that an animal can learn are connections between stimuli and responses. Until recently, there were few who stood out against such S-R theorizing, though even in the 1940s and 1950s there were distinguished exceptions such as Edward Tolman and Karl Lashley. Moreover, even when S-R theories were at their height, many interesting phenomena were discovered that could not readily be fitted into such an impoverished view of the mental capacities of animals, and that drove theorists to propose increasingly *ad hoc* and implausible mechanisms, including internal and unobservable responses.

Contemporary Animal Learning Theory is appropriately entitled, since Anthony Dickinson ignores both the follies and the findings of the past: indeed, out of the hundred or so references cited only three are dated before 1967, although many of the phenomena with which he deals (for example, overshadowing, blocking, latent inhibition and secondary reinforcement) were discovered long before. Since there has been a great deal of recent work on these phenomena, his failure to trace the origins of the discoveries is perhaps no great matter and does not affect the explanations he proposes. Dr Dickinson maintains, with Locke, that learning is based on the detection of associations between events (including as one kind of event the animal's own responses). He acknowledges that animals may learn more complex rules (for example, always to select the odd shape of a set of shapes presented), but his book is concerned only with the laws of association learning: these turn out to be much more complicated than anything foreshadowed by Locke.

Most S-R theorists have supposed that responses are learned solely because they are rewarded. One amongst many results that reveals the inadequacy of this view is the following. An animal is trained to press a bar to obtain sucrose pellets; it is then given sucrose outside the apparatus and afterwards made violently sick, a procedure that produces an aversion to sucrose. If replaced in the bar-pressing apparatus, the animal will no longer press the bar. According to Dr Dickinson, it has learned the connection between an action (bar-pressing) and a specific event (availability of sucrose pellets) and once the pellets become aversive, it ceases to press the bar.

To illustrate the kind of theorizing with which Dr Dickinson deals, consider the

Contemporary Animal Learning Theory. By Anthony Dickinson. Pp.177. Hbk ISBN 0-521-23469-7; pbk ISBN 0-521-29962-4. (Cambridge University Press: 1981.) Hbk £12.50, \$27.50; pbk £3.95, \$9.95.

following finding. If an animal is exposed to a tone that is always followed by shock but receives shock equally often when the tone is not present, it learns little or nothing about the connection between tone and shock. From a survival view point this is clearly sensible since there is in fact no causal connection between tone and shock. Nevertheless, all the older theories of learning predict that the animal will learn that shock follows tone. It is hard to believe that animals calculate the relative frequencies with which the shock occurs in the presence and in the absence of the tone. R.A. Rescorla and A.R. Wagner have put forward an ingenious resolution of this problem. They assume that there is a finite limit to the total strength of the associative bonds formed between any set of stimuli and an event associated with those stimuli. Now in the experiment described, the stimuli that are associated with the shock include not merely the tone but stimuli from the apparatus in which the animal is placed: moreover, the latter stimuli are always associated with the shock whereas the tone is only associated with it on half of its occurrences. At first, associative bonds between the shock and both tone and apparatus will be formed. But when the combined strengths of these bonds reaches its limit, the associative bonds between apparatus and shock will continue to gain in strength on trials on which shock is given without the tone, and the strength gained will be deducted from the strength of the bonds between tone and shock. Eventually the association between tone and shock will be reduced to zero. It is as though someone has a finite sum of money to invest, and once all of it has been invested, any one investment can only be increased by reducing another.

This kind of theory explains many other findings: for example, it explains why, if a strong and a weak stimulus are presented simultaneously and always followed by shock, the animal learns only the association between the more salient stimulus and the shock even though it will learn the association between the weaker stimulus and the shock perfectly well if that stimulus is presented on its own in association with shock.

It was said of E.C. Tolman who produced an earlier but much vaguer version of association theory that "he left the

animal lost in thought" — he could not explain what led an animal to act on the knowledge acquired through association. Dr Dickinson tries to overcome this problem by assuming that animals can make simple inferences: from a knowledge that bar-pressing leads to food, they can infer that they must press the bar to obtain food when hungry and hence they act. Unfortunately, not all responses are determined so logically. There are experiments that suggest that both animals and people sometimes learn to make a response to the stimulus and continue to make it even when the contingencies between events change. Someone who has been in a car accident may be unable to control his fear when next in a car, even though he knows that his recent experience has not changed the probability of a further car accident. It remains obscure what determines whether an organism will learn a direct connection between a stimulus and a response with the result that the response is involuntarily produced whenever the stimulus occurs.

Although recent theories of animal learning are more rigorous than previous accounts, they are not easy to grasp. *Contemporary Animal Learning Theory* is not for the casual reader, but it is well organized and carefully argued and is the best introduction to a field that ten years ago appeared to many to be moribund. □

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Crustacean 2 in 1

Thomas E. Bowman

The Biology of Mysids and Euphausiids. Advances in Marine Biology, 18. By J. Mauchline. Pp.677. ISBN 0-12-026118-9. (Academic: 1981.) £38.60, \$89.

THIS volume contains separate reviews of two orders of Crustacea that were formerly joined in the order Schizopoda. Because of similarities in their compound eyes, it has been argued recently that the Schizopoda should be revived, but at present the Mysidacea are assigned to the superorder Peracarida and the Euphausiacea to the superorder Eucarida (but not to the decapods as Mauchline states in his preface).

The justification given for combining

the two works in one volume is the "continuing interest in both of them by modern workers". But experts on euphausiids tend to deal with plankton, whereas students of mysids tend to be specialists on the benthos — few zoologists (Mauchline apart) have demonstrated a competence in both groups. Thus many students of mysids who will need Mauchline's indispensable work will have to purchase the euphausiid part, which may be of only marginal interest to them, and vice versa. Could not the two parts have been issued separately at more reasonable prices?

Part I, "The Biology of Mysids", is a major contribution which will be an invaluable aid to mysid biology. The taxonomic sections are especially useful. Chapter 2 contains an illustrated key to the world genera, not heretofore available. Some of the line illustrations suffer from muddy printing of crowded spines and setae, and instead of the traditional couplets the key sometimes requires the reader to choose among as many as seven items. Nevertheless, with its aid one should have little difficulty in identifying the genus of an unknown mysid. Keys to species are not provided, but Appendix I updates the 1977 World List of Mauchline and Murano, listing all known species, and Appendix II guides the reader to the most useful references for each genus.

Chapters 3–13 concisely summarize various aspects of the structure, behaviour and life history of mysids. Mauchline reviews and comments on the literature, and does not hesitate to add new material or to re-work published data. When space limitations preclude detailed discussion of a subject, pertinent references are cited.

A reviewer traditionally takes note of some flaw or error in the book under discussion. This has proven difficult, but I will fulfil the requirement by pointing out that within the suborder Mysida statocysts are lacking in *Spelaeomysis* and *Stygiomysis*, not only in the Petalophthalmidae.

Part II, "The Biology of Euphausiids", is a supplement to the 1969 volume of the same title in the same series by Mauchline and Fisher. The earlier book, summarizing information available up to 1968, is the standard primary reference work on the 85 species of Euphausiacea, major constituents of the marine zooplankton. The present volume reviews the progress made since that time.

Both parts are followed by addenda covering the most recent research, and each part contains an extensive bibliography. Taxonomic and subject indexes conclude the volume.

Many of us will use this book frequently in the years ahead, and Dr Mauchline deserves our gratitude for making such a useful tool available. □

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Reactions in the biggest retort of all

Geoffrey Eglinton

Marine Organic Chemistry: Evolution, Composition, Interactions and Chemistry of Organic Matter in Seawater. Elsevier Oceanography Series, 31. Edited by E.K. Duursma and R. Dawson. Pp.521. ISBN 0-444-41892-X. (Elsevier Scientific: 1981.) \$105, Dfl. 215.

THE oceans have been with us for most of the history of this planet and certainly over the last 4,000 million years. In this time, life has appeared, burgeoned, evolved and become the major factor in determining our present planetary environment. Continents have come and gone, wandered and pirouetted around the world's surface, all the time with erosion contributing chemically to that vast, dilute, low-temperature reaction medium — the waters of the world's oceans.

The chemistry of the seas is a matter of great wonder. Such a vast volume of water and an almost unimaginably complex dilute soup of organic compounds of largely unknown composition, constantly being contributed to, consumed, decomposed and recycled by marine organisms — a daunting prospect for researchers, even with modern chromatographic and spectrometric techniques, but progress is being made.

Duursma and Dawson have brought together in this book some 16 chapters, covering a wide range of topics, including the organic compounds present in marine organisms, non-living dissolved and particulate matter, the decomposition of organic matter, organometallic interactions, and the intriguing "skin" of the sea where organic compounds concentrate and photochemical processes occur. Most of the chapters are informative reviews rather than critical assessments. Indeed, it would have been useful to have had listings of important areas of lack of knowledge.

Another criticism is that most of the chapter topics are somewhat unconnected, even though the treatments given are excellent in their own right. Of course, it is always a difficult task to persuade busy people to write detailed and informative review essays but it is especially difficult to get over the next hurdle, that of coaxing the authors to integrate their chapters with one another. Again, the depth of coverage given to topics is uneven in this volume. Thus, the chapter on natural, halogenated, organic compounds by William Fenical is a detailed review of what has so far been found in marine organisms and of the distribution processes — liberation, transport, biodegradation and so on. Organic sulphur compounds are also given particular consideration (by Wolfgang Balzar) but the only other group of compounds to receive special attention are the hydrocarbons, in a chapter written by Alain Salot.

I recommend this book: there is much of considerable value for marine chemists, as both basic analytical data and the processes taking place are reported in some detail. However, it whets the appetite rather than satisfying as a complete meal — probably the right thing to do at this stage in our understanding of the subject. □

Geoffrey Eglinton is Professor of Organic Geochemistry in the School of Chemistry, University of Bristol.

Models in the sand

M.J. Chadwick

Arid-Land Ecosystems: Structure, Functioning and Management, Vol. 2. International Biological Programme, 17. Edited by D.W. Goodall. Pp.605. ISBN 0-521-22988-X. (Cambridge University Press: 1981.) £50, \$110.

THIS volume completes the IBP study of arid ecosystems. The first volume (for review see *Nature* **280**, 522) described the climate, soils, geomorphology, hydrology and the biotic components of arid zone ecosystems, mainly from a structural point of view, and then went on to deal with the processes controlling ecosystem functioning. Consideration of this latter component is expanded in the work under review, but to it are added ecosystem dynamics and a section on the management of arid lands. This allows the development of models to be pursued, and in many ways it is these chapters that form the most interesting part of the book. The International Biological Programme provided the stimulus for considerable advances in ecosystem modelling and, to a large extent, arid ecosystems benefited perhaps more than others from this, due to the rather fragmentary and somewhat primitive conceptual framework of knowledge of this biome when the programme began. The task is far from complete, but the stage is now set for the testing of such models against field data. It is to be hoped that the extent of arid land degradation will not be too great to prevent the successful application of such validated models to problems of arid land management.

There is a sense in which this volume is a disappointment, but not because of any inherent deficiencies in the studies it describes, the data it produces or the overall presentation of the work. It is because much of the work has appeared in various forms already, over the years that have elapsed since the termination of the

programme in June 1974. Indeed the manuscripts of some chapters of the book date from October 1973 (others being submitted up to February 1980). It is not surprising, therefore, that some authors took steps to publish accounts of part of their work in advance of this volume. Under the circumstances the editors are to be congratulated for producing such a well-rounded and coherent report.

Interactive processes in desert ecosystems are dealt with in detail (soil-vegetation-atmosphere; plant-plant; animal-animal; plant-animal) and these lead on in a logical fashion to an overall account of the population dynamics of ecosystems in deserts. Arid land ecosystems are so variable, temporally and spatially, that these systems test to the limit the theories developed in this field. Wagner stresses this in his account of patterns of fluctuation, mechanisms of equilibrium and constraints limiting density for a range of desert species. Much of this work is incorporated into the models of arid ecosystem dynamics (Goodall), spatial effects in modelling (Noy-Meir) and the simulation of plant production in arid regions (van Keulen and de Wit). In all of this, Goodall contends that attempts "to understand the dynamics of an ecosystem without taking biological individuality into account are doomed to failure". His chapter, and that of Noy-Meir, are succinct essays on the realities of modelling ecosystems.

The limitations, both scientific and geographical, on the use of models for land management strategies in the arid zone are evident from the final chapters of the book. These deal with management for domestic livestock forage, for dryland and irrigated crops, for recreation and tourism, of water resources and the social aspects of managing arid ecosystems. A final chapter attempts a synthesis. The real synthesis is for the years ahead. □

M.J. Chadwick is a Reader in the Department of Biology, University of York.

Unearthing the past

Patty Jo Watson

A Complete Manual of Field Archaeology: Tools and Techniques of Field Work for Archaeologists. By Martha Joukowsky. Pp.630. Hbk ISBN 0-13-162164-5; pbk ISBN 0-13-162156-4. (Prentice-Hall: 1980.) Hbk \$29.95, £22.45; pbk \$14.95, £11.20.

MARTHA Joukowsky has produced a good, sturdy field manual in an attractive format. The author's training and experience are principally in Old World Mediterranean archaeology, and the book reflects this bias. Although New World prehistory is

not entirely unrepresented, the author is not at home with American archaeology nor the terminology and techniques characteristic of it.

As the title indicates, emphasis is on field recording and — to a lesser extent — recovery techniques. There are chapters on surveying, stratigraphy, record forms and record books for a variety of data categories, and on the analysis of pottery and several other artifact types. But there are also short chapters on archaeological staff, ethics in archaeology, dating processes, publication, fieldwork opportunities, financial aid and a longer discussion of field conservation. With the exceptions noted below, the text is well written and clear with abundant line drawings illustrating the descriptive material.

No such volume can be or should be entirely encyclopaedic, but there are some unfortunate omissions or inadequacies. A summary of dry-screening methods is provided (written by Junius Bird), but there is very little attention given to water-screening and only a brief discussion of flotation/water separation techniques. Botanical recovery in general is more briefly covered than that of faunal and human skeletal remains. Several definitions in the glossaries provided here and there in the text are cursory, misleading or vague. For example, on p.171 mud-brick is listed synonymously with adobe and pisé, then defined as unbaked or sun-dried brick. But many archaeologists use pisé to mean architecture of coursed, sun-dried mud, or (sometimes) beaten earth. The New World synonym is puddled adobe; a common Near Eastern synonym is *tauf* (Arabic) as distinct from *libn*, which is mud brick. The definitions of layer, level and locus on the same page are also rather confusing. Similarly, many of the definitions given for various chipped stone tool types (pp.320-321) are vague or misleading; they were not prepared by a lithics specialist.

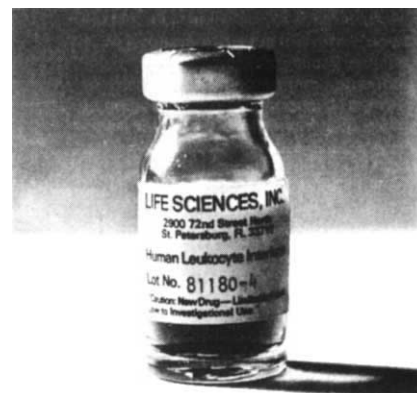
Another weakness is the bibliography. The subjects treated are categorized in a somewhat idiosyncratic way ("Culture Studies" is the least comprehensible heading, but there are others that are also arbitrary, such as "New World Mounds"), and references are often missing or seem randomly distributed among the headings.

Although there is no detailed discussion of problem definition or research design, the few paragraphs devoted to these vital topics (p.38) are clear and well phrased.

In spite of the inadequacies of the book for use by New World prehistorians, every professional archaeologist should be aware of it and most of them should own it. By and large, it is a worthy successor to Wheeler's *Archaeology from the Earth* (Penguin, 1954) and Kenyon's *Beginning in Archaeology* (Praeger, 1953). □

Patty Jo Watson is a Professor in the Department of Anthropology at Washington University, St Louis. Her most recent book is Archaeological Ethnography in Western Iran (University of Arizona Press, 1979).

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CORRESPONDENCE

Continued from page 506

In case some legalistic quibble is to be put on the world "good", Milton clears the point in a famous passage.

"Good and evil we know in the field of this world grow up together almost inseparably; and the knowledge of good is so involved and interwoven with the knowledge of evil, and in so many cunning resemblances hardly to be discerned, that those confused seeds which were imposed upon Psyche as an incessant labour to cull out, and sort asunder, were not more intermixed. It was from out the rind of one apple tasted, that the knowledge of good and evil, as two twins cleaving together, leaped forth into the world. And perhaps this is that doom which Adam fell into of knowing good and evil, that is to say of knowing good by evil. As therefore the state of man now is; what wisdom can there be to choose, what continence to forbear without the knowledge of evil? He that can apprehend and consider vice with all her baits and seeming pleasures, and yet abstain, and yet distinguish, and yet prefer that which is truly better, he is the true wayfaring Christian."

F.W. COUSINS

London SW1, UK

SIR — I must voice my grave concern that, in the influential editorial pages of *Nature*, reasoned argument has given way to the emotional outburst of your comment "A book for burning?" (24 September, p.245). Amid many heated adjectives you condemn Dr Sheldrake's *A New Science of Life* as "the best candidate for burning there has been for many years" because (a) his claim that it can be tested is "preposterous", and (b) the theory is incomplete regarding the "nature and origin" of the morphogenetic fields postulated by Sheldrake and "the means by which they are propagated". The second reason is, you say, "more serious", adding that "hypotheses can be dignified as theories only if all aspects of them can be tested".

This second objection, if it were partial grounds for making a publication a candidate for burning, would prevent the publication of any hypothesis until it had been articulated to its last detail — a sure method of stifling all innovation.

For the first objection, (a) above, three arguments are advanced: (i) the experiments are time consuming; (ii) it would be possible to explain away negative results; (iii) no grant-making agency would support the experiments. Argument (i) would condemn all research into inheritance, not just that proposed by Sheldrake; argument (ii) applies in principle to any experiment, but is in this case vacuous since Sheldrake clearly states that he would regard failure as disproof; and argument (iii) is equally empty in its appeal to "higher authority" without any indication as to why no agency would support the experiments.

I share *Nature's* concern expressed in the comment that the public should not gain the impression that science contains irrational elements. But the way to combat this impression is by displaying rationality.

C.J.S. CLARKE

University of York, UK

SIR — In a leading article (*Nature* 24 September, p.245) you reject Dr Sheldrake's morphogenetic fields as "pseudo science" on the grounds that he does not prescribe their nature or origin, or discuss how their laws of propagation might be discovered. But the properties of heat, light and sound were investigated long before there was any understanding of their true nature, and electricity and magnetism originally had exactly the status that you criticized in the hypothetical water-divining example. Were such investigations pseudoscience?

You claim that hypotheses can be dignified as theories only if *all* aspects of them can be tested. Such a criterion would bar general relativity, the black hole and many other concepts of modern science from being regarded as legitimate scientific theories.

The discussion of Dr Sheldrake's proposed experiments and their falsifiability is rendered void since it assumes *a priori* that the experiments will fail.

The rapid advances in molecular biology to which you refer do not mean very much. If one is on a journey, rapid progress on the way implies neither that one is close to one's destination, nor that the destination will be reached at all by continuing to follow the same road.

By referring to "self-respecting grant-making agencies" you show a concern not for scientific validity but for respectability. The fundamental weakness is a failure to admit even the possibility that genuine physical facts may exist which lie outside the scope of current scientific descriptions. Indeed, a new kind of understanding of nature is now emerging, with concepts like implicate order and subject-dependent reality (and now, perhaps informative causation). These developments have not yet penetrated to the leading journals. One can only hope that the editors will soon cease to obstruct this avenue of progress, and instead encourage reviews of the field.

B.D. JOSEPHSON

University of Cambridge, UK

Non-random survival

SIR — Barrie Pearson (*Nature* 3 September, p.6) states that non-random survival (Flew's revised formulation of the principle of natural selection, *Nature* 16 July, p.192) is "an empirically empty concept if the range of possibilities the non-random survivors were selected from is unknown".

Fortunately, and contrary to what Pearson appears to believe, the "range of possibilities" is known in quite a number of cases in which the variation amongst juvenile members of an animal population can be shown to be far greater than the variation amongst the adults. This has been demonstrated for populations of snails, lizards, sparrows, fossil cave bears and humans: in some cases the selection is stabilizing and in other cases it is directional. Natural selection would thus seem to have a satisfactory empirical basis.

The depressing thing about the evolutionist/creationist controversy is not so much the heated arguments about the "status" of the theory of evolution as the fact that the creationists are attempting to sell as scientific a theory which most scientists had abandoned as inadequate long before Darwin's theory was ever thought of: even Cuvier found that one

creation was hardly enough! As a theory attempting to explain as much as possible of the way in which the world of living organisms arrived at its present state, without resorting to metaphysical explanations, the neo-Darwinian theory wins hands down over the creationist theory. Thus Pearson's "creatures of reason" are quite right to prefer neo-Darwinism to creationism if they want a theory based — as far as possible — on physical rather than metaphysical grounds. And this, after all, is the essence of the scientific enterprise.

Odense, Denmark

MIKE ROBSON

Names for proteins

SIR — We were glad to see the reference by Brian F.C. Clark (*Nature* 6 August, p.491) on the prospects for standardizing nomenclature for an index of human and other mammalian cell proteins separated by two-dimensional gel electrophoresis. Lest disproportionate resources be spent on a catalogue of specialized nomenclature, we would urge that parallel projects yielding precise chemical identification (where feasible) are essential.

We believe that it is well within present competence to characterize the perhaps 30,000 protein structures produced in measurable amount in each species and to tabulate a good deal of accessory information about them. However, the ultimate method for identifying chemical compounds, which is understood by people in diverse fields, is through covalent structure. Proteins are no exception. Determination of only a few residues of an unknown protein can usually serve to identify the group to which it belongs¹ if the sequence of a closely related structure or homologue in another mammal has already been determined. Identity of sequence also serves to clarify the many situations in which proteins of almost identical structure have rather different properties in gels and in which immunological tests are misleading.

It is not generally realized how extensive is the collection of protein sequences already elucidated. We have just completed the integration of data from the *Atlas of Protein Sequence and Structure*, Vol.5, and its three supplements with the more recent data, including that derived from nucleic acid sequences². There are more than 1,600 entries in the collection. (Within an entry we describe information on structures less than 5 per cent different from one another, such as alleles.)

We have also grouped the data into superfamilies and families: sequences in the same superfamily are significantly similar using statistical tests, whereas sequences in the same family within a superfamily typically are less than 50 per cent different from one another. In the current collection there are almost 500 superfamilies and 750 families; over 140 of these superfamilies and 219 families contain at least one mammalian sequence. There are some 200 entries containing human sequences.

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1. Dayhoff, M.O. & Orcutt, B.C. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2170-2174 (1979).
2. *Protein Sequence Data Tape 81* (National Biomedical Research Foundation, Washington DC, 1981).